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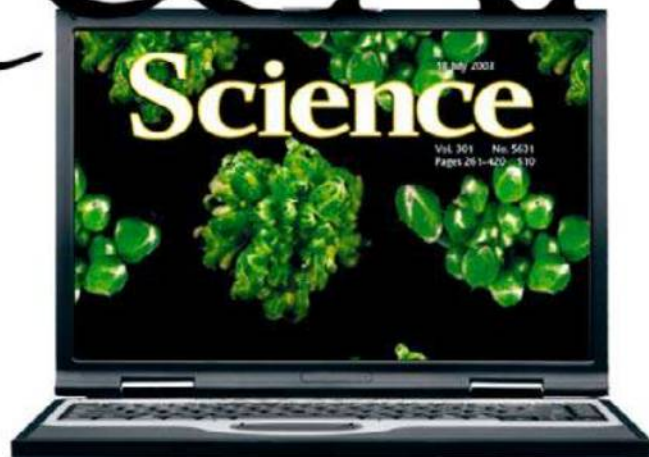
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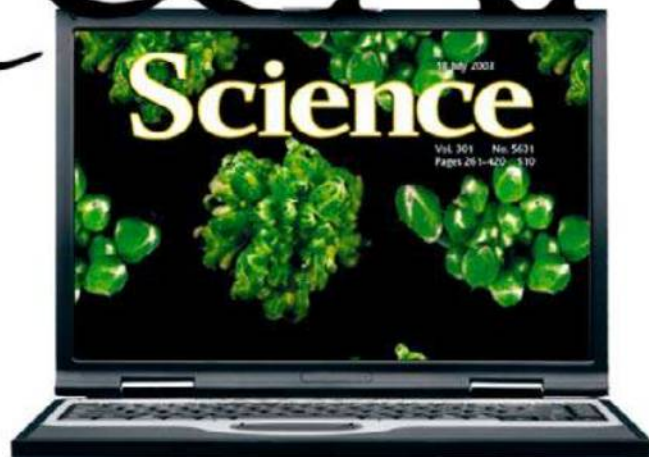
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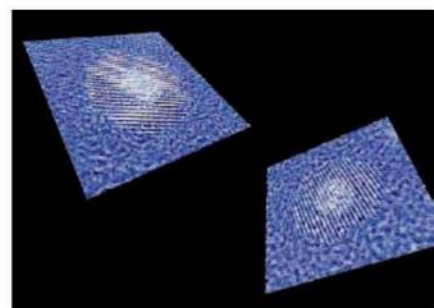
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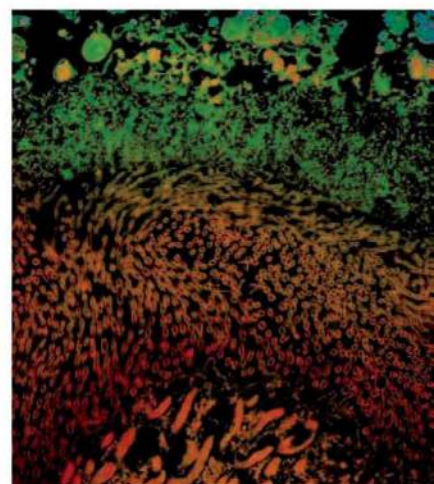
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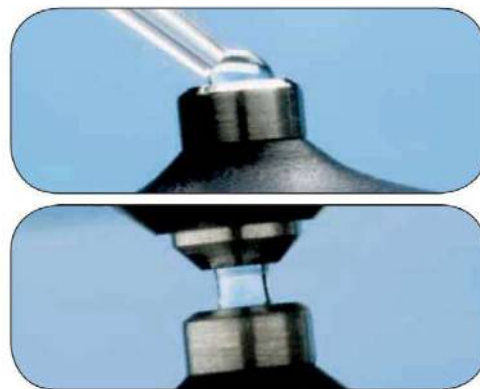
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Structural Basis of Preexisting Immunity to the 2009 H1N1 Pandemic Influenza Virus
R. Xu et al.

An epitope conserved between the 1918 and 2009 pandemic flu viruses explains age-related immunity to the 2009 virus.

10.1126/science.1186430

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10.1126/science.1184246

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RESEARCH ARTICLE: Gain-of-Function Enhancement of IP₃ Receptor Modal Gating by Familial Alzheimer's Disease-Linked Presenilin Mutants in Human Cells and Mouse Neurons

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Specification of T cell lineage in the thymus is controlled by the timing and strength of signaling of the tyrosine kinase Zap70.

SCIENCE CAREERS

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A Shifting Drug Industry Means New Opportunities in Translational Research

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Pharmaceutical companies are hiring researchers for their early drug-development programs.

Structuring a Career Around Gallium Nitride

E. Pain

An early success has put the career of materials scientist Rachel Oliver on a fast track.

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COMMENTARY: Taking Risks with Translational Research

I. Mills

Neither a purely corporate nor purely academic model is entirely appropriate to translate genetic data to understand complex diseases.



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RESEARCH ARTICLE: Cross-Neutralization of 1918 and 2009 Influenza Viruses—Role of Glycans in Viral Evolution and Vaccine Design

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RESEARCH ARTICLE: A Conformal, Bio-Interfaced Class of Silicon Electronics for Mapping Cardiac Electrophysiology

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ADVANCING SCIENCE. SERVING SOCIETY

Trophic Trade-Offs >>

There have been many attempts to document and explain the effects of predators on plant biomass in so-called “trophic cascades.” Theory suggests that fast-growing plants are relatively undefended and suffer more from herbivory, which implies a functional trade-off between investment in traits relating to growth and defensive strategies. **Mooney *et al.*** (p. 1642; see the Perspective by **Hambäck**) compared responses to fertilization and aphid predators in 16 milkweed species. As predicted, interspecific variation in the strength of top-down control in terms of a tradeoff with growth was observed.



Platinum-Free Diesel

The efficiency advantages inherent in diesel-based combustion engines are counterbalanced by the production of pollutants such as nitrogen oxides (NO_x). Currently, expensive precious metals, such as platinum, are required to remove these pollutants. **Kim *et al.*** (p. 1624; see the Perspective by **Parks**) show that a strontium-doped perovskite catalyst, prepared from more abundant (and cheaper) elements, may help to lower the cost of NO_x treatments and thus ultimately make diesel a more cost-effective automotive fuel. Under conditions realistically simulating exhaust streams, the catalyst rivaled platinum in accelerating NO_x decomposition.

Iron Exposure

The macrocyclic heme motif coordinates iron ions in proteins and plays a widespread role in biochemical oxidative catalysis. **Bezzu *et al.*** (p. 1627) prepared crystals in which analogous iron-centered macrocycles were aligned in pairs. The outer faces of the pairs exposed the iron ions to vacant cavities, where ligand exchange could take place; the inner faces were bound together by rigid bridging ligands lending the crystals structural integrity. The stability and high porosity of these crystals lend themselves to potential catalytic applications.

Preventing Radiation Damage

Inside a nuclear reactor, long-term exposure to radiation causes structural damage and limits the lifetimes of the reactor components. **Bai *et al.*** (p. 1631; see the Perspective by **Ackland**) now show, using three simulation methods able to cover a wide range of time and length scales,

that grain boundaries in copper can act as sinks for radiation-induced defects. The boundaries are able to store up defects, in the form of interstitials, which subsequently annihilate with vacancies in the bulk. This recombination mechanism has a lower energy barrier than the bulk equivalent, and so provides a lower-cost route for the copper to self-heal.

Fermion Behavior in an Optical Lattice

Due to their extreme tunability, optical lattices loaded with fermions and bosons are expected to act as quantum simulators, answering complicated many-body physics questions beyond the reach of theory and computation. Some of these many-body states, such as the Mott insulator and the superfluid, have been achieved in bosonic optical lattices by simply changing the characteristic depth of the lattice potential wells. Now, **Hackermüller *et al.*** (p. 1621) describe an unusual effect in an optical lattice loaded with fermions: When the strength of the attraction between the fermions is increased adiabatically, instead of contracting, the gas expands in order to preserve entropy.

Perfect Mismatch

Heteroepitaxy, or the overgrowth of one crystalline material onto a second crystalline material, is a key fabrication method for making thin

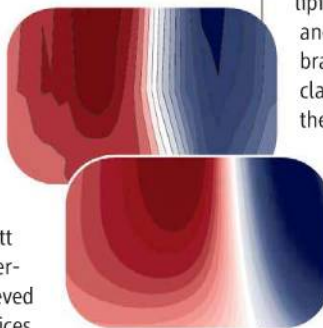
films and nanoparticles. But if the lattice mismatch between the two materials is too large or messy, fractured interfaces result. **Zhang *et al.*** (p. 1634) describe a synthesis strategy to obtain spherical nanoparticles with a core-shell architecture that does not depend on heteroepitaxy. Silver was deposited and converted to various semiconductors through a series of chemical transformations to yield structurally perfect single-crystal semiconductor shells on a gold core, despite mismatches approaching 50%.

Lipid Kinase Revealed

The lipid kinase, Vps34, makes the key signaling lipid phosphatidylinositol 3-phosphate [$\text{PI}(3)\text{P}$] and has essential roles in autophagy, membrane trafficking, and cell signaling. It is a class III PI 3-kinase, a class against which there is currently no specific inhibitor. **Miller *et al.*** (p. 1638) now describe the crystal structure of Vps34. Modeling substrate binding and combining structural data with mutagenesis suggests a mechanism in which Vps34 is auto-inhibited in solution, but adopts a catalytically active conformation on membranes. Structures of Vps34 with existing inhibitors might allow for the generation of inhibitors with high affinity and specificity.

Mosquito Double Act

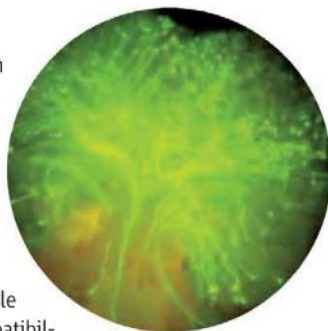
Peroxidase/dual oxidase (duox) systems act in concert to catalyze the nonspecific formation of dityrosine bonds, which cross-link a variety of proteins. Knowing that these reactions are involved in fine-tuning insect immune responses, **Kumar *et al.*** (p. 1644, published online 11 March) investigated how the peroxidase/duox system in malaria-vector mosquitoes pro-



ffects the gut flora by modulating midgut antibacterial responses. Generating immune reactions resulted in a loss of mosquito egg viability, but modulating host responses allowed malaria parasites to persist among the surviving commensal flora. The peroxidase/duox system appears to promote dityrosine bond formation between proteins across the surface of midgut epithelial cells to form a layer that inhibits immune recognition and mediator release. Interference with the formation of this layer might provide a target for mosquito and malaria control.

The Making of the Males

Most plants have a hermaphroditic mating system with flowers with both male and female function. However, in some cases, species are invaded by a sex-specific sterility factor. When female sterility factors invade a population, it results in a mating system called androdioecy. Theoretically, these female sterile (male) individuals should occur at low frequencies because of their reduced reproductive capacity. However, some species in the olive family have a greater than expected frequency of males. **Saumitou-Laprade *et al.*** (p. 1648) show that, for one species, males were able to reach high frequencies because of the retention of a self-incompatibility factor within hermaphroditic individuals. In this case, hermaphroditic individuals can only mate with individuals outside of their incompatibility type, reducing their available mating partners, whereas males are able to mate with all hermaphrodites. This explains how, contrary to theory, high frequencies of males can exist within populations.



A Pathway to Leukemia

Leukemia is initiated and maintained by a small number of self-renewing cells called leukemia stem cells (LSCs), which share properties with hematopoietic stem cells (HSCs), the self-renewing cells that produce healthy blood cells. **Wang *et al.*** (p. 1650) studied mouse models of acute myelogenous leukemia (AML), a disease that is often refractory to existing therapies. Activation of the Wnt/ β -catenin signaling pathway was required for efficient oncogene-mediated conversion of HSCs into LSCs. This pathway is among the most well studied signaling pathways in cell biology, setting the stage for testing of β -catenin signaling antagonists in preclinical models of AML.

Heart Cell Signaling in 3D

A healthy heart relies on the proper transduction of cellular signals through the β_1 - and β_2 -adrenergic receptors (β ARs), which are located on the surface of the heart's muscle cells (cardiomyocytes). The surface of these cells resembles a highly organized series of hills and valleys and it has been unclear whether this topography plays a role in the β AR signaling events that are critical to cell function. **Nikolaev *et al.*** (p. 1653, published online 25 February; see Perspective by **Dorn**) monitored the cyclic adenosine monophosphate (cAMP) signals generated by the β ARs in living cardiomyocytes. In cells from healthy rats and from rats with heart failure, the β_1 ARs were localized across the entire cell surface. In contrast, the spatial localization of the β_2 ARs differed in healthy and failing cells. In healthy cardiomyocytes, the β_2 ARs resided exclusively within surface invaginations called transverse tubules, thereby producing spatially confined cAMP signals, whereas in failing cardiomyocytes, the β_2 ARs redistributed to other cell surface areas, thereby producing diffuse cAMP signals. Thus, changes in the spatial localization of β_2 AR-induced cAMP signaling may contribute to heart failure.

Shelterin the Ends

The ends of linear chromosomes suffer two problems: They cannot be replicated to their termini, resulting in loss of terminal sequences; and they can be mistakenly sensed as DNA double-strand breaks, activating DNA repair pathways that can result in serious genome derangement. These problems are solved by the addition of telomeres, repeat sequences at the ends of chromosomes, which are shielded by a protein complex called shelterin. **Sfeir *et al.*** (p. 1657) show that the mouse Rap1 protein, which is part of the shelterin complex and which binds to a second shelterin protein called TRF2, helps prevent telomeres undergoing unscheduled homologous recombination. Such recombination could threaten telomere integrity by generating sequence exchanges between sister telomeres resulting in critically shortened telomeres.

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Food Security for a Billion Poor

THERE ARE AT LEAST 1 BILLION POOR PEOPLE LIVING WITH CHRONIC UNDERNOURISHMENT, and the United Nations (UN) Millennium Development Goal of substantially reducing the world's hungry by 2015 will not be met. The developing world's poor are experiencing the effects of higher commodity prices, and declining agricultural productivity growth is exacerbating the problem. Next week, leaders in science and society will convene in Montpellier, France, for the first Global Conference on Agricultural Research for Development (GCARD 2010) to organize sweeping changes in global agricultural research. The meeting follows major reforms of the Consultative Group on International Agricultural Research (CGIAR), endorsed in December 2009. CGIAR's new business model is meant to more effectively address food security, focusing on people, results, and efficiency. "Mega Programs" (now called "Themes") will deliver research outputs to achieve scaled-up impacts on poverty, and a new fund will harmonize donor contributions to support CGIAR's 15 research centers. But the total global investment in public-sector agricultural research is 20 times greater than that of CGIAR. How to better harness this critical resource (along with private-sector investments) for worldwide poverty reduction will be a major focus for GCARD.

The main battlegrounds for poverty reduction are Asia and Africa, where 97% of the world's food-insecure reside. Although international food prices have declined from their peak in 2008, they have remained higher than the trend in developing countries, reflecting a supply/demand imbalance. Contributing to the problem, cereal yields growth has declined. According to the Intergovernmental Panel on Climate Change, a 2°C increase in temperature could lead to a further 20 to 40% fall in cereal yields, mostly in Asia and Africa. Lifting a billion people out of poverty and feeding an extra 2.3 billion by 2050 will require increasing cereal production by 70%, doubling the output of developing countries.* In sub-Saharan Africa, where more "ultrapoor" live, developing technologies to boost productivity is especially difficult because of greater threats from pests and diseases, poorer soil, and drought. In addition, Africa's R&D establishments are small compared to those of South Asia—half had fewer than 100 scientists in 2000. Compared to Latin America, Africa has less than half the rural roads per hectare, 1/40th the capital per farmer, and 1/50th the rural electricity supply per worker.† Despite some success with maize, cassava, and some horticultural crops, few African countries have experienced a Green Revolution.

Agricultural development requires a combination of enabling policies; secure land rights; and farmers' access to knowledge, technologies, and markets. Some technologies, such as new plant varieties or those that enable more efficient use of water and nutrients, can be obtained from international sources. But innovations involving natural resource management or the involvement of women have no universal blueprints and must be developed to suit particular conditions. Hence, restructuring CGIAR with sharper research priorities that bring in partner countries is a hopeful sign.

Public investment in agriculture is critical. Only Brazil, China, and India have boosted research expenditures as a share of agricultural gross domestic product; but it may now be increasing elsewhere, after two decades of decline because of complacency among policy-makers. The 2009 pledge of the G8 countries of \$20 billion in new aid to food and agriculture over the next 3 years, with a focus on Asia and Africa, should help, as will an anticipated expansion of South-South partnerships. The goal of the GCARD 2010 meeting is to transform the global architecture of agricultural research over the next several years, as an essential complement to the CGIAR reforms. The stakes are high: Without this change, we may face a billion more hungry on our planet.

— Uma Lele

10.1126/science.1189247

*World Summit on Food Security, Food and Agriculture Organization of the UN, Rome, Italy, 16 to 18 November 2009.

†J. Schmidhuber, J. Bruinsma, G. Boedeker, in *Proceedings of the Expert Meeting on How to Feed the World by 2050*, Food and Agriculture Organization of the UN, Rome, Italy, 24 to 26 June 2009.



Uma Lele is an author of *Transforming Agricultural Research for Development* for GCARD and a former senior advisor for the World Bank.





SUSTAINABILITY

Weighing Water

In comparing competing sources of energy, many recent analyses have focused on relative conversion efficiencies and associated greenhouse gas emissions. However, other potentially limiting factors also contribute to the value of a given approach. Based on the prediction that fresh water will become one of the most limited resources in the future, Mulder *et al.* estimated the energy return on fresh water input (for production and processing) across a range of energy technologies. One of the more striking outcomes of the analysis is that the most efficient petroleum-based energy source (diesel fuel) yields over two orders of magnitude more energy per volume of fresh water used than does biomass. Such a vast difference in return on water invested suggests that policies striving to replace fossil fuels with biomass resources—their many other appealing characteristics notwithstanding—may exacerbate the increased burden on a global fresh water supply already stressed from the higher agricultural demands of a more populated world (though feedstock shifts may relieve some of this pressure). Solar and wind technologies show potential advantages in this context. — NW

AMBIO 10.1007/s13280-009-0003-x (2010).

APPLIED PHYSICS

Spinning into View

The efficiency of electrical devices is compromised considerably by Joule heating. In an attempt to thwart this effect, spintronics seeks to manipulate the electron's spin rather than its charge. This manipulation is perhaps best exemplified by the spin-transfer torque effect, in which passing a spin current from one ferromagnetic layer into another through an intervening nonmagnetic film (the so-called magnetic tunnel junction) causes the relative polarization of the two ferromagnetic layers to switch from parallel to antiparallel, or vice versa. This switching process has been challenging to characterize on a single-shot basis as the signal (change in conductance) is quite weak. Now, in a single-event experiment, Cui *et al.* resolve not just the switch itself but the smaller oscillations of the conductance leading to a switch (reflecting the increasingly intense precession of the spins). As the measurements are performed at room temperature and thermal effects are important in assisting the switching process, the observed dynamics may be more generally applicable to nonlinear systems in warm environments. — JS

Phys. Rev. Lett. 104, 97201 (2010).

EVOLUTION

Splitting on the Edges

Explanations of the species richness on coral reefs have often inferred diversification processes from large-scale patterns. In contrast, quantitative phylogenetic studies, based on thorough taxonomic and spatial sampling, can document the speciation events responsible for regional diversity patterns. Applying this approach to the abundant and colorful *Calcinus* hermit crabs, Malay and Paulay have constructed mitochondrial and nuclear gene phylogenies for 56 species (9 undescribed) and mapped their distributions. Differences in color patterns follow species boundaries and evolve rapidly, indicating a likely role in species recognition. Most speciation in the genus has occurred peripherally, in remote areas. All of the younger species pairs are narrowly allopatric, and molecular clock analyses imply that sister species require at least 2 million years (and usually much longer) to develop secondary sympatry. There are a few major ecological shifts between sister species, but environmental preferences are conserved across most speciation events (niche conservatism). These hermit crabs' strong preference for oceanic areas and substantial ability for dispersal have led to diversity peaks in the Mariana Islands and in southeast Polynesia rather than in the Indo-Malayan triangle (the locus of maximum marine biodiversity). The example of *Calcinus* serves as a reminder that different groups, having their individual histories and ecologies, should not be expected to show identical biogeographic patterns. — SJS

Evolution 64, 634 (2010).



PLANT BIOLOGY

Partners in Reproduction

Production of the next generation in plants follows a more tortuous path than the corresponding process in animals. In *Arabidopsis*, the next generation is made up of both embryo and endosperm, which are produced in two separate fertilization events by separate sperm (albeit from the same meiotic generation). Pillot *et al.* have analyzed how the transition from an initial reliance on maternal RNAs to subsequent zygotic transcription occurs for both next-generation tissues. Although the zygote can coast on maternally supplied transcripts for a while, the endosperm cannot and becomes self-reliant from the moment of fertilization. These differences can be attributed to the epigenetic status of the chromatin. Analyses of DNA methylation and histone modifications show that the differences in the female gametes—the egg and central cells—are established late in gametogenesis. — PJH

Plant Cell 22, 10.1105/tpc.109.071647 (2010).

Inspired Thinking for Universities of the New Millennium

Osaka University is one of the world's leading research-based universities. Here, President **Kiyokazu Washida** offers an inspiring insight into the factors underscoring the success of Handai—an innovative university with a global presence.

Osaka University—or Handai as it is known colloquially—traces its roots to two unique places of study: the Kaitokudo, an academy founded by five local merchants in 1724, and the Tekijuku school, established in 1838 by Koan Ogata, a pioneer of European medicine.

The Kaitokudo and Tekijuku are the foundations and inspiration of the 'Handai style' that places emphasis on cutting edge research, creation of new research fields, respect for liberal arts at the graduate school level, and contributions to society by active university-industrial collaboration.

Now in the 21st century, the university is a world-class institute consisting of over 30,000 students and staff, 11 undergraduate schools, 16 graduate schools, 5 research institutes, 4 libraries, 2 hospitals, and 24 collaborative-use facilities dispersed over the Suita, Toyonaka, and Minoh campuses. Quite fitting for the university motto of 'Live locally, grow globally', Handai has 74 inter-university academic exchange agreements with universities in over 20 countries, and notably, four Overseas Centers for Education and Research, located in San Francisco, USA; Groningen, The Netherlands; Bangkok, Thailand; and Shanghai, China.

Kiyokazu Washida is the 16th president of Osaka University. He has the distinction of being the first president with an arts background, with a prolific research portfolio covering the fields of philosophy and ethics. Washida has received many prestigious awards including the Suntory Prize for Social Sciences and Humanities in 1989, the Kuwabara Takeo Prize in 2000, and a Medal with Purple Ribbon from the Emperor of Japan in 2004.

"My predecessors were scientists," says Washida. "But science is a form of art. So I do not think of myself as being as different from them as some people may think." Washida stresses that his own background gives him a highly objective view of scientific research. "Noh is a highly stylized classic form of Japanese theatre where the characters wear masks," explains Washida. "And in Noh the term '*riken-no-ken*' refers to the ability of an actor to look at himself from the viewpoint of the audience, that is, objectively. This ability is important for university management, as well as other professions. I encourage my staff and students to cultivate this skill."



INNOVATIVE, GLOBAL ENGLISH LANGUAGE PROGRAMS

Osaka University is one of 13 core Japanese universities selected to participate in the 'Global 30 Project for Establishing Core Universities for Internationalization' program, or G30, by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in 2009. One of the major aims of the participating institutes is to double the number of overseas students by 2020. Each university is allocated a budget for five years, with which to recruit and educate 3000 to 8000 international students.

"As part of our 'Global 30 Action Plan' we have developed new and highly specialized courses which will be given in English," says Washida. In addition to ongoing programs, the Osaka University curriculum will see the launch of four new courses by the year 2011.

- Human Sciences undergraduate degree program, starting in April 2011
- Chemistry/Biology Combined Major Program, undergraduate degree program, starting in October 2010
- Special Integrated Science graduate school course, starting in October 2010
- International Physics graduate school course, starting in October 2010

Internationalization is a two-way process, and independently of this new program, domestic students studying at Handai are given an opportunity to study at partner universities overseas for up to 12 months.

THE MEANING OF GLOBALIZATION

"Modern science has been and will continue to be truly global," says President Washida. "Generations of scientists have been able to validate each other's ideas and experimental results irrespective of their location—science crosses all borders."

So what is globalization? "In the modern era, I believe that globalization refers to major changes in education," says Washida. "History shows that education essentially teaches young people the skills required to manage the society they live in. And education has been based on local 'values' of a particular country and its culture; so education has historically been a local exercise."

But modern society is highly interconnected and necessitates global thinking. "The food we eat, household goods and so on are often produced in other countries," says Washida. "A good education in the 21st century must be transferable internationally—I refer to this as 'inter-locality'."

The G30 program is a means of addressing issues related to global education by sending students at Osaka University abroad, and welcoming overseas students to study in Japan. "I would like our students to spend at least six months abroad during their stay here," says Washida. "Nurturing multilingual students, with the ability to respect and understand other cultures, is one of my major goals."



COMPETITIVE SOCIETY AND THE MEANING OF 'MATSU'

Universities must prepare students to cope with the fast pace and intensely competitive society triggered by the proliferation of information technology. Washida offers a unique philosophic view about competition and industrial trends with reference to the word 'wait,' or 'matsu' in Japanese.

"Modern society is based on competition, with a tendency to be constantly looking forward, a focused 'prospective view,'" says Washida. This can have a downside, however, in that competition can result in too much emphasis moving forward, such that the value of careful consideration is lost.

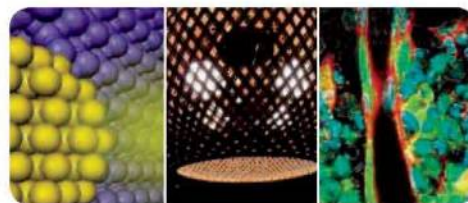
Washida adds that these competitive and rapid actions can be counterproductive, and not necessarily a rich and enlightening experience for all concerned—especially in academia—where careful thought requires time, and cannot be rushed.

"Such 'pro'-based action in traditional forms of agriculture and fishing industry would not yield favorable results," says Washida. "For example, fruit that is harvested too early is bitter, and fishermen must be patient and wait to catch their fish. Waiting is an extremely important part of these industries. My point is that too much competition is just as bad as too little. That is the significance of 'matsu', or waiting. In the case of a university, one of my roles is to find an appropriate balance for encouraging innovation and discovery."



Left to right: Vice President Kiichiro Tsuji, in charge of the G30 program at Osaka University; the Life Sciences Library; and President Kiyokazu Washida.

"Whether we consider fashion, medical care or education, we do not need a heavy textbook on philosophy to understand what is happening around us. We just need to be more observant." — Kiyokazu Washida



CUTTING EDGE RESEARCH FOR THE FUTURE

Osaka University is a research-based institute that emphasizes 'basic', 'exciting', and 'responsible'.

With a view to strengthening its research infrastructure for the future, Washida has initiated the establishment of world-class research centers. Some recent high profile, multi-million dollar research initiatives include:



- The Immunology Frontier Research Center (IFReC), led by world renowned immunologist **Shizuo Akira**, with a mission of 'whole body imaging of the human immune system'. The IFReC was selected in 2007 as one of the highly prized World Premier International Research Center Initiative programs initiated by MEXT.



- **Toshio Yanagida** is collaborating with nine other groups and six companies on the 'Yuragi Project', where the concepts of biological fluctuations—yuragi—are being applied to information networks, and robotic machines and systems for improved energy conservation, lower cost, and greater security and comfort.



- **Satoshi Kawata** is director of the Photonics Advanced Research Center, and leads a project on 'evolving from electronics to photonics'. The aim of the project is to exploit the properties of light to manipulate nanomaterials such as carbon nanotubes and biomolecules.



- **Tomoji Kawai** is head of a new project: Ultra-fast Single-Molecule DNA Sequencing, Ultra-Low Concentration Virus Detection, and Ultra-Sensitive Biomolecule Monitoring. The aim is the development of the ultimate biochip using single molecule analysis technology.

'CLINICAL PHILOSOPHY' AND THE FUTURE

Osaka University is internationally recognized as having made important contributions to the arts, medicine, physical sciences, and engineering. What does the future hold? "I would like to encourage our faculty and students to work on defining problems that the human race must address for a safe and prosperous society," says Washida. "Simply responding to the social needs of tomorrow is not enough. We must address issues of the 'day after tomorrow'."

Washida captures the essence of the critical reasoning process required to achieve these goals with the term 'Clinical Philosophy', a phrase that refers to connecting fundamental philosophical ideas to modern society. "This simply means taking a closer look at our everyday surroundings," says Washida. "Whether we consider fashion, medical care or education, we do not need a heavy textbook on philosophy to understand what is happening around us. We just need to be more observant."

Washida was elected president in August 2007, and he has a clear vision for the future. "I want to see our students acquire a high level of literacy in a wide range of languages," says Washida. "The Global 30 program will be a catalyst for this. Also I would like Osaka University to be an integral part of our local, and needless to say global, society. Finally, I want Osaka University to be the birthplace of outstanding ideas and discoveries that capture the imagination, and resonate world-wide. If asked 'where was this discovered', I would like to hear, 'that it originated at Osaka University'. That's the *Handai* style."



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Geek Chic

In celebration of National Science and Engineering Week in the United Kingdom, lab coats made by fashion designers went on show in London last week. "Labcoats = Safety," according to London-based designer Oknah Somit, who says this creation "brings a focus on the safety issue whilst the cut of the tail coat adds a classic and formal element."

The Genes of Finns

Curious about their origins, willing to provide DNA samples, and the subject of reproductive near-isolation for centuries, the Finnish are a gold mine for population geneticists and disease gene hunters.

More than 40,000 Finns have donated to the Finnish Gene Atlas, a project initiated by Leena Peltonen, who died on 11 March. Scientists offered a preview of the findings last week in Helsinki at the launch of the Institute for Molecular Medicine Finland (FIMM). They reveal several distinct subpopulations in different parts of the country, likely caused when subsets of the population headed out to settle new territory.

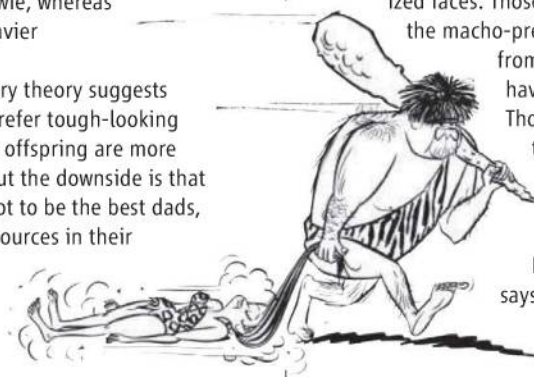
"Finns are really at a crossroads between East and West, and there is possibly a northern influence as well," says geneticist Olli Kallioniemi of the University of Helsinki, director of FIMM. The project has also challenged a theory, long held by anthropologists, that Finns are related to Hungarians because of similarities in their languages. There's "little evidence that Finns would be any more closely related to Hungarians than to other Central European populations," says FIMM geneticist Samuli Ripatti. Having

distinct, but closely related, Finnish populations should be useful in finding disease genes, says geneticist Mark Daly of Harvard Medical School in Boston. If researchers find, for example, that two closely related populations have significantly different incidences of coronary artery disease, he says, "this might allow us to find the few places in the genome where [they] differ and figure out which is relevant."

When Macho Appeals

Why do some women go crazy for androgynous men like David Bowie, whereas others swoon for Javier Bardem?

One evolutionary theory suggests that women may prefer tough-looking guys because their offspring are more likely to survive. But the downside is that manly men tend not to be the best dads, investing fewer resources in their offspring. So women should go more for he-men when local health risks are high. A study published last week in the *Proceedings of the Royal Society B* supports that idea.



For the study, led by Lisa DeBruine, a psychologist at the University of Aberdeen in the United Kingdom, some 4500 women from 30 different countries in Europe and the Americas went to a Web site (faceresearch.org) and noted their preferences between pairs of 20 different white male faces, some of them digitally manipulated to increase or decrease masculine features. The researchers also ranked the relative health of the women's countries using statistics from the World Health Organization.

The less healthy a woman's country was, the more likely she was to prefer the masculinized faces. Those at the high end of the macho-preferring scale came

from Brazil, also ranked as having the worst health.

Those who tended more toward the girly men were from Belgium and Sweden, the healthiest.

It's a "novel finding," says psychologist Fiona Newell of Trinity College Dublin in Ireland. But Newell

cautions that more masculinized faces tend to look older, a feature related to "social fitness" and wealth.

DOES SONAR DISRUPT DEEP DIVERS?

Beaked whales are the world's most extreme divers, reaching depths of 1888 meters to dine on squid. To avoid getting the bends—a buildup of nitrogen bubbles in body tissues—they need to come up slowly, so dives can take as long as 90 minutes.

But some whales, particularly young ones, have been so disturbed by anti-submarine sonar during naval exercises that they shoot up *en masse* and end up dead on beaches. This has happened in at least six cases in the Canary Islands, leading the Spanish Navy to move exercises farther offshore.

The U.S. Navy also holds sonar exercises off the coast of Hawaii. No mass strandings have been reported there, but some researchers warn that local whales may be suffering from the same effects as those in the Canaries. In a paper published in *Marine Mammal Science* this month, biologist Robin Baird of the Cascadia Research Collective in Olympia

and graduate student Meghan Faerber of the University of Wales, Bangor, in the United Kingdom say predatory sharks, strong currents, a natural habitat farther offshore, and other factors could be concealing whale casualties. "It would make sense to at least ban sonar within 45 kilometers of the Big Island," Baird says. The U.S. Navy so far hasn't changed the position stated in a 2006 report: that "any significant behavioral response" to the sonar from the Hawaii whales is "extremely unlikely."



RESEARCH FUNDING

Health Bill Backs Evidence-Based Medicine, New Drug Studies

Buried within the 2400 pages of the landmark health care reform bill passed by the House of Representatives this week and signed by President Barack Obama are several provisions that touch on clinical research. Two are aimed at determining which therapies work best and identifying

Health (NIH) and the Agency for Healthcare Research and Quality (AHRQ) (*Science*, 27 November 2009, p. 1183).

Some lawmakers wanted to put the new institute within AHRQ, a unit of the Department of Health and Human Services (HHS), which has been the federal home for CER.

Creating a new organization, they argued, would be duplicative and wasteful. Others felt independence would encourage involvement from “stakeholders” such as doctors and patients. “Some feel it’s good to get a fresh take outside existing structures,” says Steven Pearson, president of the Institute for Clinical and Economic Review at Harvard Medical School in Boston. The new institute will set a research agenda and award contracts mainly through NIH and AHRQ. Funding for the institute will rise to \$150 million in 2012, to be sup-

plemented after that by a trust fund drawn from fees on health insurance.

A less-noticed section of the health legislation creates a new translational research program within the NIH director’s office aimed at drug development. Called the Cures Acceleration Network (CAN), it is the brainchild of Senator Arlen Specter (D-PA). CAN will give out grants and contracts of up to \$15 million a year to companies, academic researchers, and patient groups to help bridge the “valley of death”: the gap between the initial discovery of a drug and the lab and animal studies a developer needs to conduct to obtain regulatory approval for clinical trials.

The NIH director will decide who gets the competitive awards, which must go for “high need cures” or high-priority therapies

that are unlikely to be developed by the commercial market. CAN will also have an advisory board, at least one-third of whose 24 members will be from patient advocacy groups. Amy Comstock Rick, executive director of the Parkinson’s Action Network in Washington, D.C., which pushed for CAN, says her group’s disease illustrates the problem: Companies haven’t been interested in Parkinson’s because the market isn’t that big and a lack of biomarkers makes it hard to tell if treatments are working. “We believe it’s time for the NIH to expand its piece of the pipeline a little bit,” Rick says.

It’s not clear yet who will pay for CAN. Although the bill authorizes up to \$500 million a year, nothing has been appropriated. Some biomedical research groups worry that money would be shifted from existing NIH programs to fund CAN. “We are concerned in a time of limited resources that it could constrain research budgets,” says Carrie Wolinetz, spokesperson for the Federation of American Societies for Experimental Biology in Rockville, Maryland, which did not take a position on CAN. The bill also elevates NIH’s Center on Minority Health and Health Disparities to an institute.

Finally, the bill puts into place a widely supported proposal to shed light on the payments that drug and medical-device companies make to physicians and hospitals. Starting in 2013, the so-called Physician Payments Sunshine Act provisions require that such companies file annual reports to HHS listing all payments or other “transfers of value” to doctors and hospitals of more than \$10 per event or \$100 total per year. The information will be posted in a public online database. The legislation is a response to a Senate investigation that found such payments are often not disclosed and may be influencing prescribing decisions and biasing the results of clinical studies.

An earlier House version of the provisions would have covered all biomedical scientists, including those without a medical degree. But supporters say the language in the final bill is an important start and could be expanded later. It “will better protect patients and will help restore trust in our health-care system,” says Allan Coukell, director of the Pew Prescription Project, a proponent of strong disclosure rules.

—JOCELYN KAISER



Done deal. House Democrats cheer passage of the health insurance reform bill, which contains a few initiatives in medical research.

researchers’ financial conflicts. A third, which has flown under the radar until now, would fund a new push in drug development at the National Institutes of Health.

Most directly tied to health care is a provision that focuses on comparative effectiveness research (CER)—evidence-based studies that compare the value of medical treatments, such as two different drugs, or a specific drug versus surgery. Proponents hope that these studies will improve the quality and lower the cost of health care by identifying the best treatments. The bill creates an independent, nonprofit Patient-Centered Outcomes Research Institute to conduct this research. It picks up on the \$1 billion in 2-year funds for CER that last year’s stimulus package gave to the National Institutes of



The neglected
female rodent

1571



California
compromise

1574

SWINE FLU PANDEMIC

What's Old Is New: 1918 Virus Matches 2009 H1N1 Strain

The “novel” H1N1 swine influenza virus that last year caused the first human pandemic in 4 decades has one feature that is hardly novel: Its surface protein, hemagglutinin (HA)—which spikes cells and starts an infection—closely matches the HA in the H1N1 virus responsible for the 1918 pandemic. Separated by 91 years, the two strains of the highly mutable virus ought to be vastly different. This newfound similarity answers many mysteries about the 2009 pandemic, including why it largely spared the elderly. The new findings from different research groups also suggest intriguing explanations for how the 1918 influenza virus has evolved since it swept across the globe in several waves, killing more than 50 million people by the winter of 1919. And the investigators are proposing provocative—some say far-fetched—vaccination strategies to preempt future pandemics.

Influenza researchers not involved with the new studies say they pull together several concepts about the relationship between influenza, the immune system, and different species that have been gaining ground. “I really find this a fascinating story,” says Rino Rappuoli, head of vaccine research at Novartis Vaccines & Diagnostics in Siena, Italy. “It’s the lesson of evolution.”

One study published 24 March in *Science Translational Medicine* shows that even though nearly a century separates the widespread circulation of the two viruses in humans, mice given a vaccine against the 1918 strain produced antibodies that “neutralized” the novel 2009 strain. When the team flipped the experiment and used a 2009 pandemic vaccine in mice, the immune response stopped the 1918 virus. “We kind of did a double take,” says virologist Gary Nabel, head of the Vaccine Research Center at the U.S. National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland, and the lead researcher on the project. “It was an unexpected finding, but it all makes sense when you look at the data collectively.” Although he acknowledges the

limits of extrapolating from mouse to human immune systems, Nabel says in this restricted analysis of antibodies in response to proteins, “it’s a very reasonable model.”

Influenza and the human body are like opposing Cold War spies, with the virus repeatedly donning new disguises and the human immune system racing to foil each incarnation. HA is the virus’s main quick-change artist. Antibodies produced by the immune system, in turn, try to neutralize HAs by binding to them, blocking the virus from entering cells. As a rule, influenza

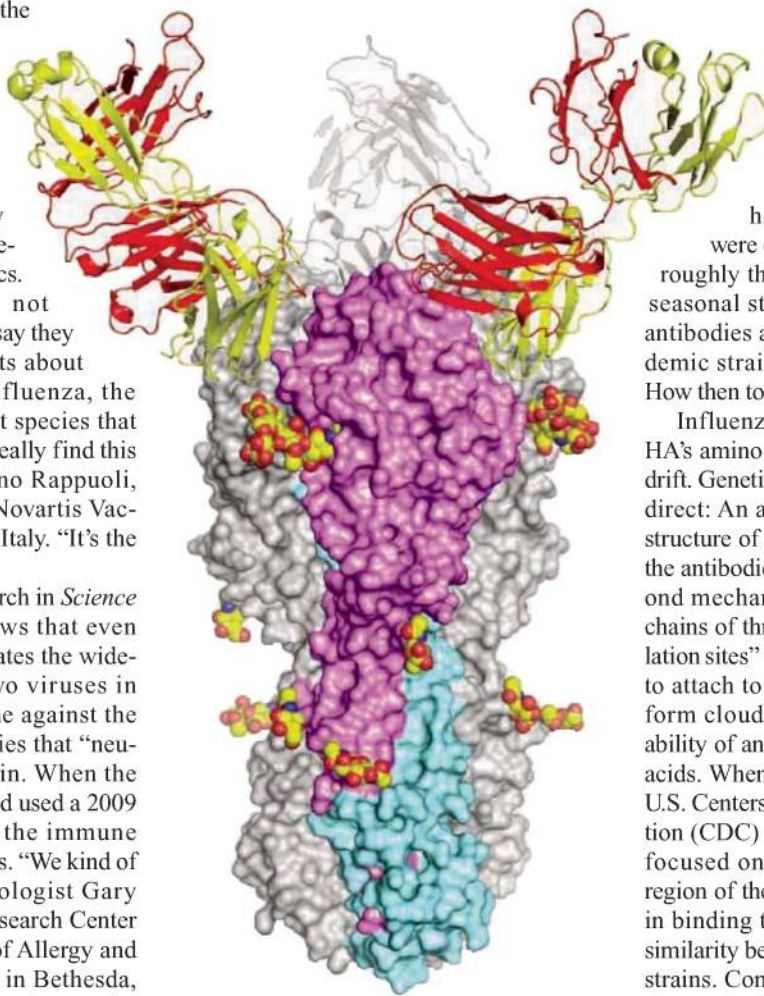
viruses change so quickly that a vaccine against a regular “seasonal” strain circulating one year may have little impact against a similar strain a few years later. Yet the HA proteins on the 1918 and 2009 pandemic viruses look remarkably similar in close analyses done in both Nabel’s study and a separate one published online this week by *Science* that includes x-ray crystallographic data (www.sciencemag.org/cgi/content/abstract/science.1186430). These two reports also clarify the evolution of seasonal strains in the decades between the two pandemics.

The two studies focus on the top part, or the head, of the HA, which is the business end of the protein when it comes to the infection process.

Each research group calculated that the amino acids in the head of the two pandemic HAs were only about 80% similar, which is roughly the divergence seen between two seasonal strains. This would suggest that antibodies against the 1918 and 2009 pandemic strains would *not* cross-neutralize. How then to explain the mouse results?

Influenza foils antibodies by changing HA’s amino acids—a process called genetic drift. Genetic drift occurs in two ways. One is direct: An amino acid change can alter the structure of the protein so that the “arms” of the antibodies can’t get a good grip. The second mechanism involves sugars. Specific chains of three amino acids create “glycosylation sites” that allow sugars made by the cell to attach to the viral protein. These sugars form clouds around the HA, masking the ability of antibodies to “see” the right amino acids. When Nabel, Terrence Tumpey of the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, and their co-workers focused on the amino acids in a discrete region of the HA tip that plays a critical role in binding to cells, they discovered a 95% similarity between the old and new pandemic strains. Comparisons between seasonal and the pandemic strains in this region found less than 70% similarity.

In the second study, a team led by structural biologist Ian Wilson of the Scripps ▶



Crystal ball. The 2009 pandemic virus has the same amino acids at the tip of its HA as the 1918 strain shown here bound to an antibody (red and yellow ribbons) taken from a survivor of the 1918 pandemic.

Research Institute in San Diego, California, went further, linking the amino acid sequence analysis to the three-dimensional structure. Wilson's group crystallized the 1918 and 2009 pandemic viruses and showed that the HA heads had distinctly similar shapes. What's more, the few amino acid differences between the strains were mainly confined to one small region of the head. In an additional experiment, they took an antibody from survivors of the 1918 epidemic, crystallized it in a complex with the 1918 virus, determined the amino acids in HA used to bind the antibody, and then showed that the 2009 pandemic strain had the same amino acids. "The closest related structure that we have to the current 2009 swine flu is the 1918 structure," says Wilson,

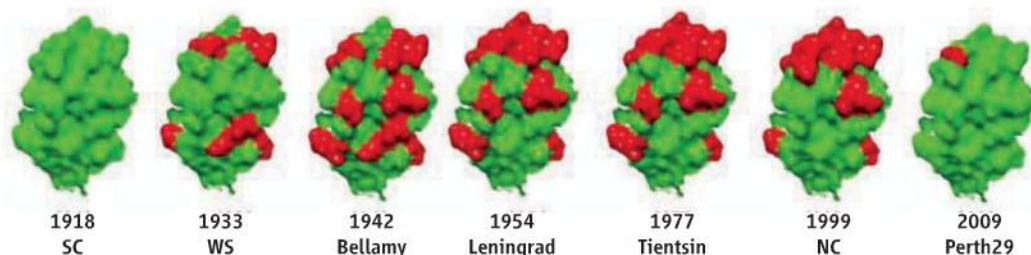
the last 91 years, we've been in one large 1918 pandemic era."

By analyzing the difference in the earliest available seasonal HAs from 1933 to 2009, Nabel's group found that some amino acid drift occurred and changed the structure of the HA head, but after that the bald virus started accumulating new glycosylation sites. Nabel posits that the bald 1918 virus could tolerate only a limited number of amino acid changes that altered its structure. "At a certain point, there's a fitness cost for adopting a new mutation, so the virus says, 'What else can I do?'" says Nabel.

In a perspective he co-authored in *Science Translational Medicine* about the study, Novartis's Rappuoli says people exposed to the bald 1918 virus and its sugar-

since 1918 is because viral evolution differs dramatically in humans and other species. Humans live many decades, creating long-term relationships between the immune system in individuals and the influenza viruses they encounter during their lifetimes. "The virus is pushed to evolve quickly in humans if it wants to survive," says Rappuoli. Not so in pigs and birds, which are short-lived species—especially those on farms—that can pass influenza viruses to humans. The H1 in pigs thus has had little pressure to mutate and has remained frozen in evolution, says Donis, who calls swine "a warm freezer."

Rappuoli thinks a clearer understanding of the relationship between influenza viruses in humans and other species could



Sugar on top. Descendants of the 1918 virus dodged antibodies by mutating (red) the tips of the HA to change shape and hold glycans, but the 2009 pandemic strain (far right) turned back the clock.

who also analyzed sequences from many other influenza viruses that have circulated in humans between those two pandemics. "The papers come to similar conclusions about why some people are more resistant to the current swine flu."

Both the Wilson and the Nabel studies show that the HAs of the two pandemic strains also look markedly different from seasonal viruses when it comes to sugars. All seasonal strains have at least two glycosylation sites on the top of their HAs, whereas both the pandemic strains are bald. "The absence of glycosylation at the top of these molecules is making a huge difference in the immune response," says CDC virologist Ruben Donis, who was not involved with the study.

The new studies are helping to clarify how influenza viruses have used sugars in their evolution since 1918, says NIAID virologist Jeffrey Taubenberger, a leading investigator of that devastating pandemic. "All the influenza viruses in humans are descendants of the 1918 virus," says Taubenberger, who published mouse experiments 8 March online in *Influenza and Other Respiratory Viruses* that similarly show how the 1918 virus protects against the 2009 pandemic strain. "Over

free descendants that subsequently circulated for a few decades developed an immunity that later protected them from the 2009 pandemic strain. "Evolution does not necessarily bring new things," says Rappuoli. "It sometimes brings things back."

The new evolutionary insights are leading researchers to revisit assumptions about another immune-evading trick that influenza exploits called genetic shift. Influenza viruses can swap whole genes, or reassort, with different strains. There are 16 different HAs and nine different neuraminidases, which is what the H and N numbers designate. The 1957 pandemic strain was just such a reassortant, with the H1N1 becoming an H2N2. A pandemic in 1968 saw a switch to H3N2. So many researchers assumed that the next pandemic would occur with H5, H9, or some other HA that few human immune systems had seen. "No one in the flu community predicted it would be an H1," says Taubenberger. "It took everyone by surprise, including me." Basically, immunity against the 1918 H1N1 had waned enough to create a niche for another bald H1 to return.

As Rappuoli notes, one of the lessons from the 2009 swine flu pandemic is that the reason the H1 remained bald in pigs

be used to craft a new vaccination strategy to prevent pandemics. He proposes making vaccines against viruses that caused earlier pandemics by pulling out "archived" strains that have remained frozen for decades in pigs and birds. Chicken farmers might similarly be given an H5N1 vaccine to reduce the chances of that highly virulent "bird flu" strain, which does not spread well between people, adapting to humans.

Nabel offers his own forward-looking vaccine strategy. He predicts that the 2009 pandemic strain will follow the mutational path of the 1918 pandemic virus; as his paper went to press, he says he detected that process in four isolates. A vaccine could be developed that artificially glycosylates the novel 2009 H1N1 in a way that mimics glycosylated descendants of the 1918 strain. "We can look at how 1918 evolved in response to humans and preemptively take steps to contain and maybe even drive the 2009 pandemic strain out of existence," says Nabel.

Taubenberger notes that huge practical hurdles stand in the way of such "boutique" vaccines. But he, too, thinks the 2009 pandemic H1N1 may help humans outwit influenza in the long run. In particular, he says the 2009 pandemic virus might out-compete the H3N2 seasonal strain that now causes most of the deaths in the elderly. "It would be fantastic if a new pandemic virus, in which the elderly are somewhat protected, replaced a nasty seasonal virus that causes serious morbidity and mortality." Now, that would be a novel twist to the 2009 swine flu pandemic.

—JON COHEN

SCIENCE AND RELIGION

Latest Prize Bolsters Templeton's Shift to Mainstream

In 2005, molecular biologist Matthew Gibson wondered whether to accept a grant he had received from the John Templeton Foundation to study how the apparently random process of cell division leads to a predictable honeycombed pattern in the epithelium of many organisms. Gibson, then a postdoc at Harvard Medical School in Boston, knew that the foundation was interested in how order emerges from randomness, a pet theme for proponents of intelligent design (ID). If he took the grant, Gibson wondered, would he be playing into a religious agenda?

Gibson was not the only scientist to harbor such doubts about the foundation, which seeks to promote a dialogue between science and religion. In the past, Templeton has supported conferences and projects linked to the Discovery Institute, an ID think tank. But it subsequently disavowed support for the ID movement, allaying the fears of many critics. This week, the foundation took another step in that direction by awarding its annual \$1.5 million Templeton Prize to Francisco Ayala, a priest-turned-biologist who for decades has campaigned against the teaching of creationism and ID in the science classroom.

The 76-year-old Ayala, a professor at the University of California, Irvine, has sought to foster mutual respect between science and religion through lectures and writings on topics such as morality. "If they are properly understood, they *cannot* be in contradiction because science and religion concern different matters," says Ayala, a former president of AAAS (publisher of *Science*). He says the conflict has grown less intense since Templeton funds helped to launch a program in the mid-1990s called Dialogue on Science, Ethics, and Religion at AAAS, which continues to be supported by the foundation. Some scientists objected at the time, he recalls. "They said, 'What business does science have talking to religion?'" I don't think there are many thoughtful scientists who would make that point today."

However, some remain staunchly opposed to Templeton's mission. "They are using the prestige and authority of science to improve the prestige and credibility of theology," says Daniel Dennett, a philosopher at

Tufts University in Medford, Massachusetts. In his opinion, Templeton-funded discussions between scientists and religious figures do for religion what debates between ID proponents and evolutionary biologists would do for ID: "They create the perception that scientists and theologians are academic co-equals, which they are not."

One program that Dennett worried has a religious slant is the Science of Generosity initiative at the University of Notre Dame in Indiana, begun last year with \$5 million from the foundation. The program has awarded a handful of research grants, including \$250,000 to study how empathy affects charitable donation and \$400,000 to explore how generosity spreads through

ton's mission agree that the foundation does not attempt to influence the outcomes of the research and discussions it sponsors. "I am not enthusiastic about the message they seem to be selling to the public—that science and religion are not incompatible; I think there is real tension between the two," says Steven Weinberg, a Nobel Prize-winning physicist at the University of Texas, Austin, who has been an outspoken critic of religion. "But for an organization with a message, they are pretty good at not being intrusive in the activities they fund. I don't wish them well, but I don't think they are particularly insidious or dangerous."

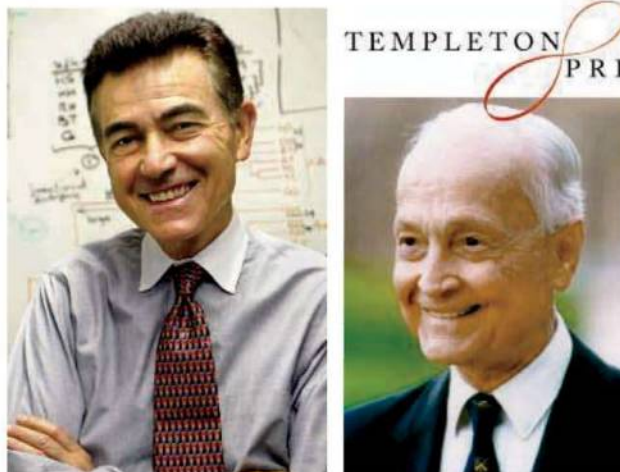
The foundation has also recognized the pitfalls of associating with the ID community after being criticized by scientists for giving a grant in 1999 to ID proponent William Dembski, a fellow at the Discovery Institute, and later to Guillermo Gonzales, an astronomer at Iowa State University who used the funds to research a book arguing in favor of ID. In a 2007 letter to the *Los Angeles Times*, Templeton's former vice president for communications explained that "[i]n the past, we have given grants to scientists who have gone on to identify themselves as members of the intelligent-design community. We understand that this could be misconstrued by some to suggest that we implicitly support the move-

ment, but this was not our intention at the time, nor is it today."

Barbara Forrest, a philosopher at Southeastern Louisiana University in Hammond, says that "Templeton realized that the relationship was a liability to their mission."

Gibson says he decided to accept the foundation's money "after poking around and finding nothing fishy." Now a researcher at the Stowers Institute for Medical Research in Kansas City, Missouri, Gibson admits that he may have been influenced by need. "At the time, I don't think anybody else would have funded what we were doing." But he's pleased with how things turned out, including a paper in *Nature*. "The fact that the [foundation] appreciated a philosophical element of the research—which I was neutral about—is fine with me," he says.

—YUDHIJIT BHATTACHARJEE



Awardee. Francisco Ayala (left) is the latest winner of a \$1.5 million prize from the foundation created by John Templeton.

social networks. "What they are trying to do is to paint certain topics with a holy glow," says Dennett.

Not so, says the program's director, Notre Dame sociologist Christian Smith. He says the initiative in no way presumes that generosity "is somehow God-given." The choice of the term "generosity," he explains, was to create an umbrella theme for different but related topics such as altruism and voluntary blood donation. "Most projects that we're going to fund will be about operations of the mind, social psychology, and related concepts," he says. Joseph Henrich, an anthropologist at the University of British Columbia in Vancouver, does not see a religious taint. "There may be a little bit of marketing" in how the program has been framed, he says, "but it's perfectly legitimate."

Even those who are put off by Temple-

From *Science's* Online Daily News Site

The Best Refrigerator Magnet Ever?

There are limits to just how magnetic a material can be, or so researchers thought. A compound of iron and nitrogen is about 18% more magnetic than the most magnetic material currently known, a team of materials scientists claims. If such magnets could be produced commercially, they could, for example, allow electronics manufacturers to equip computer hard drives with smaller "write heads" capable of being crammed with more information. <http://bit.ly/bestmagnet>

Mosquitoes Become Flying Vaccinators

A group of Japanese researchers has developed a mosquito that spreads vaccine instead of disease. Even the researchers admit, however, that regulatory and ethical problems will prevent the critters from ever taking wing—at least for the delivery of human vaccines. <http://bit.ly/vaccinators>



Landlubber Caterpillars Take to the Water

Adolescence is a tough time for anyone, but what if, on top of your growing pains, you had to learn how to breathe underwater? Hawaiian caterpillars take it all in stride. Researchers have discovered 12 species of Hawaiian moths whose caterpillars are equally comfortable submerged in a stream or beached on a bone-dry strip of land. The feat makes them unique among insects, and maybe even among animals, the researchers say. <http://bit.ly/caterpillars>

Nano-Gadget Holds the Salt

Water desalination plants can effectively turn seawater into drinking water, but they're hardly portable. Now a team has created a salt-removing gadget so small that hundreds of them could fit onto a penny. If researchers can scale up this invention into a working device, it could generate up to a glass of fresh water per minute using about the same amount of energy as a table lamp does. <http://bit.ly/nano-gadget>

Read the full postings, comments, and more on sciencenow.sciencemag.org.

HUMAN EVOLUTION

Ancient DNA From Siberia Fingers a Possible New Human Lineage

When ancient-DNA expert Svante Pääbo gave his colleague Johannes Krause a sample of a 40,000-year-old human finger bone from a Siberian cave, he had only one question: Was its mitochondrial DNA (mtDNA) that of a Neandertal or a modern human?

It was neither. Evolutionary geneticists Pääbo and Krause, of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, have apparently identified a new lineage of ancient human, the first time that this has been done using ancient DNA and not fossil bones.

"I couldn't believe it," Pääbo says. "I thought Johannes was pulling my leg." The complete sequence of mtDNA from the finger bone, reported online this week in *Nature*, suggests that Central Asia was occupied at that time not only by Neandertals and *Homo sapiens* but also by a third, previously unknown hominin lineage. "This is the most exciting discovery to come from the ancient DNA field so far," says Chris Tyler-Smith, a geneticist at the Sanger Institute in Hinxton, United Kingdom. "A stunning piece of work," says Terence Brown of the University of Manchester in the U.K.

The work complicates the human story, much as the discovery of the controversial *H. floresiensis*—a.k.a. the hobbit—has upset earlier and simpler views of early human migrations around the globe. If four hominins including the hobbit were alive about 40,000 years ago, "the amount of [human] biodiversity ... was pretty remarkable," says geneticist Sarah Tishkoff of the

University of Pennsylvania. For now, Pääbo's team is not naming the new lineage.

The finger bone—a phalanx whose species could not be identified—was found in 2008 at Denisova Cave in Russia's Altai Mountains, where archaeologists led by co-authors Michael Shunkov and Anatoli Derevianko of the Russian Academy of Sciences in Novosibirsk have worked for decades. The cave, which has many archaeological layers spanning about 100,000 years, has yielded both Neandertal and modern human stone tools, personal ornaments, and a small collection of hominin bones too fragmentary to be identified. The bone came from a layer radiocarbon-dated to between 48,000 and 30,000 years ago. The team ground up a 30-milligram sample and extracted and sequenced all of the 16,569 base pairs of its mtDNA genome, using new techniques Pääbo's group has successfully employed to sequence both Neandertal and prehistoric modern human DNA (*Science*, 17 July 2009, p. 252). The team compared the new mtDNA sequence with that of 54 living people from around the world, a roughly 30,000-year-old modern human from another Russian site, and six Neandertals.

They got a big surprise: Although Neandertals differ from modern humans at an average of 202 nucleotide positions in the mitochondrial genome, the Denisova hominin differed from modern humans at an average of 385 positions and from Neandertals at 376 positions. When mtDNA from chimpanzees and bonobos was added to the mix,

RESEARCH FUNDING

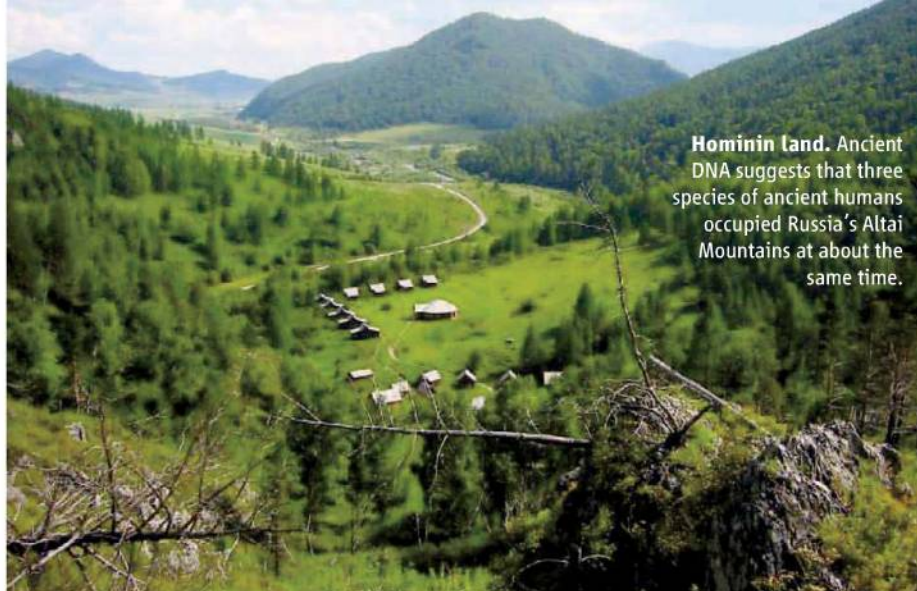
NIH Seeks Fresh Ideas on Diversity

The National Institutes of Health (NIH) is betting \$10 million that academics can find new ways to promote diversity in U.S. science.

The money will be spent on a new competition called the Director's Pathfinder Award. NIH is looking for a handful of researchers "willing to try something that would really change the game," says Clifton Poodry of the division of Minority Opportunities in Research within the National Institute of General Medical Sciences (NIGMS), which will manage the program. "What haven't we been thinking about? What type of intervention or institutional change would take us to a new level?"

The Pathfinder Award is modeled after the Director's Pioneer Award Program, begun in 2004, which generously funds individuals with potentially transformative research ideas. Pathfinder applicants must devote at least 30% of their time to efforts that will broaden the talent pool in the biomedical, clinical, behavioral, and social sciences. "We want their brains on the job," says Poodry. "It's a bit of a gamble. We're betting on the person to do something great. It's like a MacArthur grant for diversity."

Freeman Hrabowski, president of the University of Maryland, Baltimore County,



Hominin land. Ancient DNA suggests that three species of ancient humans occupied Russia's Altai Mountains at about the same time.

the researchers were able to estimate that the new hominin's mtDNA had shared a common ancestor with Neandertals and modern humans about 1 million years ago.

The team appears to have avoided contamination and other problems that have plagued ancient DNA research, says Maria-Eva Geigl of the Jacques Monod Institute in Paris, calling it a "great paper ... technically well done."

But who was this mystery hominin? The team says the date is too late for Asian *H. erectus*, which first migrated out of Africa into what is now the republic of Georgia and Java about 1.8 million years ago. And it's too early for *H. heidelbergensis*, which came on the scene in Africa and Europe about 650,000 years ago and is thought by many to be the common ancestor of modern humans and Neandertals. There's "no evidence" that these or other known species "persisted that late" in mainland Asia, says paleoanthropologist Russell Ciochon of the University of Iowa in Iowa City, although he thinks a claimed 27,000-year-old date for *H. erectus* on the island of Java remains possible.

Of course, a population of *H. erectus* may

have lingered undetected in Siberia. But if the divergence time is right, says Tyler-Smith, the new hominin cannot descend from that first migration into Asia 1.8 million years ago: Neandertals, the new hominin, and *H. sapiens* all share a common ancestor that lived 1 million years ago, presumably in Africa, according to the team. The new lineage could be related to *H. antecessor*, a species from northern Spain dated to between 800,000 and 1.2 million years ago and thought by some researchers to be the ancestor of *H. heidelbergensis*, says Chris Stringer, a paleoanthropologist at the Natural History Museum in London. Or it could represent "a pre-*heidelbergensis*, post-*erectus* dispersal" out of Africa "that we haven't picked up yet," Stringer says.

Pääbo agrees that the new lineage might represent "something that came out of Africa later" than the first *H. erectus* migration. To find out more, his group plans to try to sequence nuclear DNA from the finger bone. If they succeed, they might discover the secret identity of Hominin X. **—MICHAEL BALTER**

and a national leader in increasing minority participation in science, agrees that it's precisely those "great" individuals who can make the biggest difference in promoting diversity. "Whatever success we have achieved has had faculty involvement at its core," says Hrabowski, who has nurtured the school's successful Meyerhoff Scholars Program. "Too often, tenured faculty are not involved in these kinds of activities."

The solicitation invites ideas at all levels, from precollege through faculty members, although Poodry acknowledges that most of NIH's programs in this area target graduate and postdoctoral students. Improving undergraduate retention rates is the key to promoting diversity, says Hrabowski, and requires

innovations in teaching, curriculum, mentoring, and every other aspect of the educational process.

The deadline for applications is 4 May. NIH plans an initial screening of the six-page proposals, followed by personal interviews of the finalists. It anticipates making five 3-year awards, for \$2 million each.

The program is funded with money NIH received as part of the American Recovery and Reinvestment Act. That means NIH must commit the money by 30 September, and there is no provision for future competitions. But NIGMS Director Jeremy Berg suggested that NIH might continue the program with regular funding if the community's response is positive. **—JEFFREY MERVIS**

ScienceInsider

From the Science Policy Blog



"Why So Few?," a new report on women in science by the American Association of University Women, distills several recent reports on gender equity to provide a road map for those seeking improvements. <http://bit.ly/a5wNLN>

Three U.S. agencies have launched a joint program to predict climate change and its impacts on local scales over a few decades, fueled by \$50 million a year for 5 years. <http://bit.ly/alDpSZ>

A super-low-fuel navigation system for space flight, a software program that can recognize and analyze photos, and an exploration of mathematical representation theory snagged the top three awards at this year's Intel Science Talent Search. <http://bit.ly/8XlkwP>

Physicist Carl Wieman, a 2001 Nobel laureate and a leader in reforming U.S. undergraduate science education, has been nominated to be associate director for science in the White House Office of Science and Technology Policy. <http://bit.ly/a6q4UR>

Tim Berners-Lee and Nigel Shadbolt have received \$45 million to create the Institute of Web Science, with the goal of helping the United Kingdom extract maximum benefit from the arrival of Web 3.0. <http://bit.ly/cII3PH>

The spat over the leadership of the Royal Institution of Great Britain is headed to a showdown next month, with both fans and critics of former Director Susan Greenfield weighing in. <http://bit.ly/b6LzeA>

The U.S. National Institutes of Health is launching a new online registry that asks gene-testing companies to volunteer information on studies they've conducted on their products. The Genetic Testing Registry will be run by the National Library of Medicine. <http://bit.ly/b4Ow47>

For the full postings and more, go to news.sciencemag.org/scienceinsider.



Restoration or Devastation?

A massive South Korean project to dam and dredge four major rivers has provoked bitter opposition from scientists and environmentalists

YEJU, SOUTH KOREA—A wetland a couple of hours' drive west of Seoul may be about as close as it gets to unspoiled nature in South Korea. Baweenupgoobi's 230-plus hectares of sand dunes and gravel bars hug a bend in the South Han River, whose clear, shallow waters join the North Han and flow through Seoul. In winter, the wetland is etched with ponds and rivulets; summer rains swamp the land, as evidenced by debris lodged high in willows. The habitat offers a niche for migrating waterfowl and unusual plants, including a rare type of chrysanthemum. "These plants have evolved in harmony with seasonal flooding, and the wildlife have adapted to it," says Jeung Mingull, an ecological geneticist at Kongju National University in Gongju.

But the harmony may not last. Dams now under construction will turn the South Han into a chain of lakes. One end of Baweenupgoobi, supposedly a protected natural heritage site, has been stripped of vegetation to prepare for dredging; much of the rest will be

under water. "The government calls it 'river restoration,'" scoffs Jeung. Environmentalists mock the phrasing by calling it "river killing."

The ecological transformation extends far beyond Yeosu. Launched last November, the government's Four Major Rivers Restoration Project calls for building 16 dams, dredging 570 million cubic meters of sand and gravel to deepen nearly 700 kilometers of riverbed, renovating two estuarine barrages, and constructing bike trails, athletic fields, and parks along the waterways. At \$19 billion, it is one of the costliest engineering projects in the country's history. And it is attracting fiery opposition, notably from the Professors' Organization for Movement Against Grand Korean Canal (POMAC), a group of 2800 academics who accuse the government and supporters of twisting data and ignoring expert panel recommendations on issues such as water quality, flood control, rainfall patterns, and environmental impacts to justify a massive construction boondoggle.

Both sides agree on one point: The project will dramatically transform the Han, Nakdong, Geum, and Yeongsan rivers. Four Rivers "will be an ecological disaster," Jeung charged at a hearing in Seoul Administrative Court last month on an injunction to halt work on the South Han River. "[It] will be very beneficial for the environment," countered Jae Park, an environmental engineer at the University of Wisconsin, Madison, and a rare academic who openly supports the government position.

On 12 March, the court rejected the request for an injunction, but a suit to cancel the project is moving forward. Legal actions on the other rivers are pending. Winning even one of the suits "would be a major event in the history of the environmental movement in Korea," says Lee Sang-don, a lawyer at Chung-Ang University in Seoul.

Landscape architects

Four Rivers is a pet project of South Korean President Lee Myung-bak, a former construction company executive nicknamed "the bulldozer" for his "can do" approach to engineering projects. One of Lee's signature accomplishments in his previous role as mayor of Seoul was to demolish an elevated highway to revitalize the Cheonggyecheon River. The river is far from natural: Water is pumped in from the Han, and it flows through a concrete channel. But its walkways, landscaping, fountains, and illumination provide an oasis in what had been a grimy industrial area. When completed in September 2005,

CREDITS (TOP AND INSET): HANG JIN LEE

Unnatural development. Dams are displacing natural gravel bars (*inset*) on the South Han River.

the “restored” Cheonggyecheon was a huge hit with the public—and helped Lee win the 2007 presidential election.

One of Lee’s campaign pledges was to create a Pan Korea Grand Waterway by damming, dredging, straightening, and widening the Han and Nakdong rivers and connecting them by a canal carved through the peninsula’s central mountains. Barges, he said, would be able to move 540 kilometers between Seoul, in the country’s northwest corner, and Busan in the southeast. Lee promised that the waterway would take heavy trucks off roads, draw tourists to artificial lakes, and reinvigorate rural communities. Private investment and sales of dredged materials were supposed to cover the project’s cost.

Even before Lee took office on 25 February 2008, academics had challenged the data his team put forward to support the Grand Waterway. “It was truth versus falsehoods,” says Choe Young Chan, an agricultural economist at Seoul National University. Opposition mounted, and on 25 March, 2400 scientists, engineers, economists, and lawyers from the country’s universities converged on Seoul for the inaugural meeting of POMAC. Using members’ contributed expertise, the association pegged the project at double the cost that Lee estimated and found that sales of dredged materials would hardly make a dent in the cost. POMAC asserted that little freight moves between Seoul and Busan, and a survey of shippers turned up scant demand for a canal. The academics also questioned the project’s claimed benefits for drinking-water supplies, rural economies, and the environment.

Opponents got an unexpected boost a few weeks later when Lee announced that he would reopen South Korea’s market to U.S. beef imports, which had been banned during a mad cow disease scare. That spring, farmer and consumer groups held candlelight protest vigils in major cities. Their ire expanded to encompass other unpopular policies, including the Grand Waterway. On 19 June, Lee announced he was abandoning the canal plan.

Six months later, in December 2008, Lee unveiled a new scheme: Four Major Rivers. The “multipurpose project” will control flooding, secure water supplies, and create lakes for water sports as well as riverside parks for 1700 kilometers of bike trails and recreational facilities, says Je Hae-Chi of the Four Rivers project office. The government estimates Four Rivers will generate 340,000 jobs

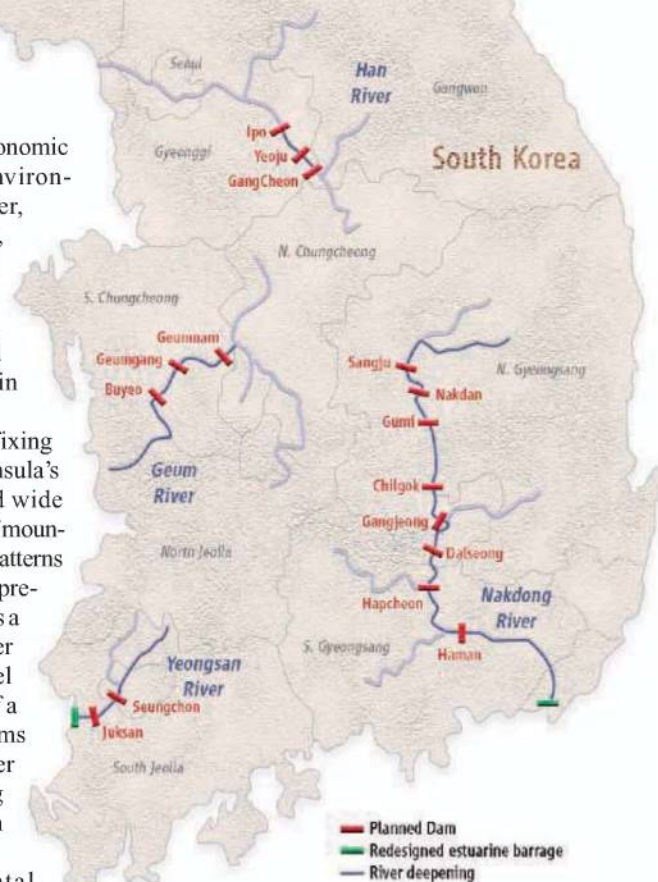
and \$35 billion in long-term economic benefits. After a 3-month environmental assessment last summer, Lee’s Grand National Party, which holds a majority in the National Assembly, pushed through enabling legislation. Lee wants the job finished before his 5-year term ends in early 2013.

Backers see the project as fixing a natural imbalance. The peninsula’s seasonally shallow rivers and wide floodplains are a consequence of mountainous geography and weather patterns that bring two-thirds of annual precipitation during the summer. As a result, during winter, low water flows expose extensive gravel bars in riverbeds—“evidence of a water deficiency,” Je says. Dams will relieve flooding and water shortages, he says, by capturing water during the rainy season for release during dry months.

Touting the environmental benefits, Lee’s administration has wrapped the project in a green mantle. River restoration is the largest component of the government’s Green New Deal, a package announced in January 2009 to counter the economic downturn with stimulus spending that promotes sustainable development (see sidebar, p. 1570). It’s “a totally different project” from Grand Waterway, says Hong Dong-gon of the Four Major Rivers project office.



On the ground. Activists use aerial photos to explain the impact of the Four Rivers plan.



Dam country. Sixteen dams will transform South Korea’s four major rivers.

To POMAC, however, the new plan is the Grand Waterway resurrected. The canal link through the mountains is missing, Choe says, but otherwise “the number of dams and their sites and the amount of dredging remains the same.”

Opponents decry what they see as unnecessary tinkering with nature. There is no question that flooding occurs on small rivers and tributaries far upstream of the planned dam sites. Instead of filling dams downstream and then building embankments and more dams on tributaries, as the government proposes, POMAC’s Park Chang-Kun, a civil engineer at Kwandong University in Gangneung, says upstream flooding could be controlled by selectively raising riverbanks and employing other watershed-management techniques. Cities along the four rivers do not face water shortages, adds Choe.

As for environmental impacts, a draft report from Birds Korea, a Busan-based environmental group, notes that Ministry of Environment data and independent surveys show that the shallow braided streams “support a higher density of waterbirds per hectare than river-impoundments.” The report concludes that habitat loss from Four Rivers will affect about 50 bird species, some considered threatened. Fish, amphibians, and reptiles will also be affected, Jeung says: “Many riverine species will disappear.”

More fundamentally, some academics believe the plan reflects outdated thinking about watershed management. “The Four Rivers Project is out of step with the way river management is evolving in the developed world,” says G. Mathias Kondolf, a geomorphologist at the University of California, Berkeley. He says planners in Europe and the United States now aim to give rivers room to meander and flood. This approach is more ecologically sound, Kondolf says, and eliminates river maintenance imposed by dredging and embankments. Project official Hong counters that based on their research and case studies of rivers in South Korea, dams and dredging “is the best solution.”

Reluctant activists

Experts who favor more ecological management of South Korea’s rivers say their findings and recommendations have been steamrolled by an administration that, in Park Chang-Kun’s view, is “distorting scientific data for political purposes.” But from the government’s standpoint, Je says, “people are in opposition for the sake of opposition.”

The list of those in opposition is growing—and includes most of the public. In a survey last October, before construction started, the Korea Society Opinion Institute reported that 26.4% of respondents wanted to see the Four Rivers Project canceled immediately; another 73.5% wanted it postponed until there was a social consensus. Dozens of South Korean and international environmental organizations have issued



Differing views. Government official Hong Dong-gon (left) sees dams as a solution for flooding and water shortages; scientist Jeung Mingull sees them as an environmental disaster.



statements opposing the plan. And the Catholic Bishops’ Conference of Korea published an instructional comic book that challenges the government on the Four Rivers Project for its “greed” and neglect of “the natural Created Order.”

Amid this wave of opposition, POMAC has played a crucial role by assessing the environmental and economic impacts of the government’s plans, holding press conferences, and supplying the expertise underpinning the lawsuits. The multitude of scientists who have joined POMAC awes like-minded colleagues in other countries. “There is a long tradition of academics working with environmental or community groups as advocates, but I have never seen any numbers like these,” says Randolph Hester, an environmental planner at the University of California, Berkeley. The self-professed activist says that for community causes in the United States, “we can get three or four

people to help us, and they might spend a week out of the year doing work for us. I’ve seen nothing like the commitment of this group.”

Even in South Korea, “such activism by academics is very unusual,” Jeung says. Politics often divide the community, but on this issue the Lee administration’s policies “have brought conservatives and progressives together,” he says. They claim to be reluctant activists. “I hate to do this; I still have to publish and teach,” Choe says. Lee Won Young, an urban planner at the University of Suwon in Hwaseong, says he got called before his university’s president to explain the time he has devoted to the cause.

The outcome of the battle over Four Rivers is up in the air. The ruling Grand National Party has blocked hearings on the subject in the National Assembly. Last month, the Democratic Party held its own hearings in which assembly member Kim Jinai outlined three scenarios for stopping the project. One is local elections in July; a trouncing of the Grand National Party could convince some assembly members to cross party lines on Four Rivers, he said. Another possibility is a construction-related disaster such as a spill of toxic chemicals that would make going forward politically impossible. The third barrier is the lawsuits. “I am confident we will win the final decision,” says Lee. But the “very complicated litigation” could last 2 years, he says. In the meantime, construction is going full throttle.

—DENNIS NORMILE

With reporting by Ahn Mi-Young in Seoul.

A ‘Green’ Blessing Raises Questions

SEOUL—South Korea’s controversial plan to transform the ecology of four rivers has become an improbable poster child of the Green New Deal movement.

In October 2008, the United Nations Environment Programme launched an initiative to encourage governments then planning recession-fighting stimulus packages to support environmentally friendly projects and forge what UNEP called a “Global Green New Deal.” Three months later, South Korean President Lee Myung-bak announced a Green New Deal under which about 80% of a \$38.1 billion stimulus package would go to eco-friendly projects. South Korea “grasped the nettle early on,” UNEP spokesperson Nick Nuttall wrote in an e-mail to *Science*.

A huge chunk of South Korea’s Green New Deal spending—originally \$10 billion, later increased to \$19 billion—was budgeted for “river restoration,” specifically the Four Major Rivers Project, an engineering scheme that critics charge is anything but friendly to the environment (see main text). Nevertheless, the Lee administration claims UNEP has given Four Rivers its seal of approval. A press release from the Office of National River Restora-

tion states: “UNEP Qualified Korea’s Epochal Green Growth Project, Korea will be newly born through the 4 Rivers Restoration Project!”

In an April 2009 UNEP report on the Global Green New Deal, economist Edward Barbier of the University of Wyoming in Laramie singled out South Korea’s green plans for special mention. But Barbier told *Science* that he did not intend to highlight river restoration “as a good project or a bad project.” Nevertheless, South Korea’s Green New Deal continued to get glowing mentions in UNEP documents. For example, an update on worldwide green stimulus spending prepared for the G20 Pittsburgh Summit meeting last September said South Korea stood out for the large percentage of its stimulus going to green investments and listed the Four Rivers project as one of the key measures.

Environmentalists seem to have finally gotten UNEP’s ear. A November draft overview from UNEP on South Korea’s Green Growth vision notes that the Four Rivers project is controversial and urges the country to assess and mitigate potential impacts on wetlands. UNEP “seemed to back off from the [previous] endorsement of the [Four Rivers] project while saving face,” says G. Mathias Kondolf, a geomorphologist at the University of California, Berkeley. The final overview is due out next month.

—D.N.

BIOMEDICAL RESEARCH

Of Mice and Women: The Bias in Animal Models

Male rodents are cheaper and easier to work with than females, but scientists worry that research done on males alone won't apply across the sexes

In 2008, Rae Silver, a neuroscientist at Columbia University, and her colleagues discovered a remarkable connection between the immune system and anxiety in mice. But something bothered her: They had done the research using only male mice. "Females have far more anxiety responses than males," she says, so she wasn't sure if her research would hold for them. And she wasn't alone in her concern. Others had found a widespread bias toward males in animal studies; almost nobody was using females in basic research.

"It's cuckoo that for diseases such as asthma, stroke, pain, immune diseases, where there are huge sex differences, people are just studying male animals," says behavioral neuroscientist Irving Zucker, a professor emeritus at the University of California, Berkeley. "It just makes no sense."

In 1993, the National Institutes of Health (NIH) Revitalization Act mandated that women and minorities be included in clinical research, because treatments had been shown to have different effects in different populations. A 2001 Institute of Medicine (IOM) report published by the National Academy Press pointed to evidence that the same was true for research using animal models: The sex of the animal can lead to qualitatively different results. And earlier this month, Silver helped organize a workshop in San Francisco, California, on behalf of the IOM's Forum on Neuroscience and Nervous System Disorders, where representatives from academia, journals, funding agencies, and the pharmaceutical industry discussed solutions to this problem of systematic sex bias in animal studies.

This bias compromises the safety and effectiveness of drugs in women, says Silver, although it's not clear exactly how much. She points to the 10 drugs that were withdrawn from the market between 1997 and 2000 because of

adverse health effects. According to a 2001 U.S. General Accounting Office report (now the Government Accountability Office), eight of them posed higher risks for women than for men, and in four of those, the risk was likely due to physiological differences. Silver suspects that lack of adequate preclinical testing in female animal models could partly explain that result.

Furthermore, obesity researcher Deborah Clegg of the University of Texas Southwestern Medical Center in Dallas says that increasing the use of female animals in research could accelerate the trend toward personalized medicine for women and ensure they're not left behind. "We'll have our own drugs, we'll have our own dosing. We'll be different, 'cause we know we are," she says.

The trouble with females

Male rats and mice have become the default animal model for many diseases because they are easier and cheaper to work with than females. Female rodents have a 4-day ovarian cycle, so researchers who use them must take daily vaginal swabs in experiments where hormones might play a role. "Otherwise, the data are uninterpretable," says obesity researcher Andrew Greenberg of the Human Nutrition Research Center on Aging at Tufts University in Boston. Scientists may also need to keep as many as four times the number of female animals as male animals to make sure their subjects are cycling in sync. And even with those precautions, the cycle may still lead to less-clear results that are more difficult to publish.

All this can add up to a lot of trouble and a lot more

money than the typical funding-agency grant provides. Many researchers say they get turned down for grants to cover the larger cost of using female animals, and many don't even bother to apply.

What is the extent of this bias, and where is it concentrated? To find out, Zucker and postdoctoral researcher Annaliese Beery recently did a survey of journal articles published in 2009 reporting results of research that used mammals. They found that only one field skewed toward females: reproductive biology. But neuroscience, pharmacology, and physiology—the very disciplines whose animal research is most likely to translate into humans—strongly

She-rats. Female rodents can be more expensive and time-consuming to work with.



skewed male. What's more, many articles across all fields failed to report subject sex at all (in immunology, 60% omitted that information), and even when both males and females were included, two-thirds of those studies failed to analyze the data by sex.

Yet many studies show that there can be surprising sex-differentiated effects in those fields. Last month, Clegg published a microarray study in the *International Journal of Obesity* that shows major sex-based differences in the gene-expression profiles of fat tissue from mice on a high-fat diet. The research was funded not by NIH, which had turned down Clegg's prior applications for research on sex-based differences, but by the Society for Women's Health Research. The society funded several top researchers to do "anything we wanted," work which "would help a lot of other scientists," Clegg writes by e-mail.

By not studying sex differences, researchers could be missing out on potential new treatments for both men and women, says Rhonda Voskuhl, director of the multiple sclerosis (MS) research and treatment program at the University of California, Los Angeles. Clinicians had observed that in women with MS, pregnancy reduces relapse of the disease by 80%. "This is an invaluable clue," Voskuhl says. "It's better than any drug we have." Based on that clinical observation, she and her colleagues tested estradiol, an estrogen produced during pregnancy, in a mouse model for MS, using both male and female animals. Encouraging lab results in the female rodents led to clinical trials testing estradiol pills as a therapy for female MS



Bench parity. Deborah Clegg says that using more female lab animals in basic research will lead to better medicines for women.

patients. The researchers expect to complete the phase II/III trials by 2013.

A similar observation—that younger men are less susceptible to MS—led to a potential therapy for men: testosterone. Voskuhl's group tested it first in mice and then as a gel in men, with promising phase II results.

Funding issues

Whatever encouraging results scientists get in the lab, drugs resulting from animal research are ultimately manufactured and sold by pharmaceutical companies, which want to make a profit. At the workshop, Morgan Sheng, vice president of neuroscience at Genentech, said that companies hesitate to spend the money necessary to test a drug candidate in both male and female animals unless the disease in question meets three rather stringent conditions: It must be known to affect men and women differently, its basic physiological mechanism must be well understood, and it must have a reasonable animal model. Otherwise, "I'm not compelled that it's a great scientific experiment to study the sex difference," he says. For funding agencies, some researchers favor implementing a policy like the Revitalization Act for animal research. "Several recent studies have demonstrated the advantage of using heterogeneous, rather than homogenous, populations in animal studies, since such studies enhance the likelihood that results generalize," Charles Mobbs, a neuroscientist at Mount Sinai Medical Center in New York City, writes by e-mail. "The NIH has recognized this regarding clinical studies, and it is certainly time a similar policy was implemented regarding animal studies."

But that probably won't happen. "I cannot foresee how a blanket policy requiring the use of male and female animals could be implemented or would work," says Vivian Pinn,

director of the NIH Office of Research on Women's Health (ORWH) in Bethesda, Maryland. "The research and how it's designed has to be based on the science of what is being studied and the availability of models."

Silver advocates channeling limited resources to areas that show clear sex differences, such as pain. "If the NIH says in the guidelines that when I study pain I have to include male and female subjects, you can bet your booties I'm going to study male and female subjects," she says—and she'll be able to ask for the extra money to do it. Without those guidelines, such studies will fall victim to the same fate as her anxiety experiment. If she wanted to rerun it in females, she thinks she would have little chance of convincing the NIH—a consistent supporter of her lab—to give her the funding.

Online

sciencemag.org

Podcast interview
with author
Corinna Wu.

Mining NIH data from large patient trials could help identify sex differences in people that would be worth studying in animals, says Richard Nakamura, scientific director of the division of intramural research programs at the National Institute of Mental Health in Bethesda. "It's unlikely that we'll find much in relatively small trials," he says. But in areas where NIH is collecting a lot of information, "we should be focusing much more on analyzing that data, and making available funds to analyze that data, to understand sex differences and outcomes."

Even if NIH does not adopt an agency-wide policy, targeted funding opportunities for studying sex differences in animal models can help a great deal, Pinn says. Even relatively small requests for applications can stimulate researchers to pursue areas they didn't before, says neuroscientist Karen Berkley of Florida State University in Tallahassee. ORWH has two such initiatives, Pinn says. It awards 5-year, \$5 million grants to establish Specialized Centers of Interdisciplinary Research in Sex and Gender Factors Affecting Women's Health, as



A vindication of the rats of women. Studying rodents of both sexes pointed Rhonda Voskuhl to potential multiple sclerosis treatments.

well as 2-year Advancing Novel Science in Women's Health Research grants for the study of sex differences.

Guidelines for journals

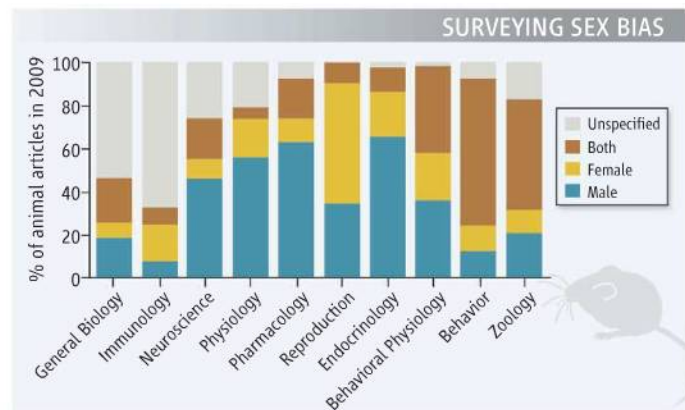
The quickest action may come from the academic journals, which are moving toward adopting a common set of guidelines for studies using animals, says Sean Murphy of the University of Washington, Seattle, chief editor of the *Journal of Neurochemistry*. The checklist of 20 items, developed by the National Centre for the Replacement, Refinement and Reduction of Animals in Research in London, would require scientists submitting manuscripts to provide details including the sex of the animals used in their experiments. A manuscript describing the recommendations has itself been submitted for publication, and from there, "it's going to be straightforward for journals to adopt the guidelines," he says. "They are sensible and will improve the quality of the papers."

Still, Murphy feels that the guidelines don't go far enough to really shine a light on the importance of sex differences. At the IOM workshop, he wondered whether journal editors should also require authors to give their rationale for studying only one sex and describe the potential implications for not studying the other.

That would at least get scientists thinking about the issue of sex bias. Currently, few graduate or medical students are trained in sex differences, and many don't know how to work with female animals. But Clegg says she answers enthusiastic questions about working with females every time she gives a talk. She says, "People go back thinking, I wonder if I should pick the females up off the shelf and actually look at them."

—CHELSEA WALD AND CORINNA WU

Chelsea Wald is a freelance science writer and editor in New York, New York, and Corinna Wu is a freelance science writer and editor in Oakland, California.



Skewed by sex. A survey of journal articles from 2009 found the strongest bias toward male animals in fields most likely to translate into humans.

BIOMEDICAL RESEARCH

Immunology Uncaged

An immunologist argues that to move beyond mice and galvanize clinical research, his field needs its own version of the Human Genome Project

With a routine blood test, your doctor can ascertain how well your metabolism handles lipids and whether you are vulnerable to heart disease. But don't expect to get a test that reveals whether your immune system is working normally or whether you are at risk for, say, autoimmune diseases. The reason: Researchers still can't define what's normal for the immune system, says Mark Davis, an immunologist at Stanford University in Palo Alto, California. Cardiologists can specify healthy levels of LDL, HDL, and triglycerides, but immunologists can't do the same for cytokines, key chemical messengers that trigger immune cells to mature, divide, attack, or perform other actions.

Researchers' reliance on mice deserves some of the blame for this ignorance, says Davis. No mouse-phobe, he keeps 400 cages of the rodents for studies of how T cells recognize pathogen molecules. But mice, says Davis, make a "lousy model" for the human immune system. The human and mouse lineages diverged some 65 million years ago, and the rodent's immune system has adapted to safeguard a small, short-lived animal that scurries around with its nose in the dirt.

However, nobody has cataloged the differences, and as a result, inconsistencies between human and mouse immunity often leave patients in the lurch, Davis says: "Hundreds of clinical trials have been based on curing mice, but almost none led to clinical treatments." Take the case of myelin basic protein (MBP). Injecting MBP into mice causes a condition similar to multiple sclerosis, which can be prevented by doses of proteins that blunt the immune reaction to MBP. But clinical trials of these protective proteins were stopped because they made some people with multiple sclerosis worse.

Failures like that have spurred Davis to call for immunology to go big science in a very human way. If enough labs combine efforts to analyze the thousands of blood samples drawn in the United States or around the world every day, a so-called Human Immunology Project could quickly amass and scrutinize data from large numbers of healthy and sick people, Davis says. Within 5 to 10 years, he predicts, "we could have the first crude benchmarks of immune function." Davis doesn't know what these benchmarks

will be—perhaps the levels of particular cytokines or the abundances of certain types of T cells—but he says researchers will probably settle on five or six variables that reflect overall immune status in people, the equivalents of LDL, HDL, and triglyceride levels.

Davis is far from the first to point out the "mouse" problem in immunology. "Studies on mice are very elegant and beautiful, but they aren't reflecting the needs of the [human] population," says Jacques Banchereau, head of the Baylor Institute for Immunological Research in Dallas, Texas.

Hoping to address this problem, Davis over 2 years ago helped to found Stanford's Human Immune Monitoring Center. HIMC analyzes blood samples mainly from Stanford clinical research labs but also from some biotech and pharmaceutical companies, says Director Holden Maecker. The center can measure levels of 50 cytokines, run microarrays to nail down gene activity, and gauge the abundance of more than 30 varieties of white blood cells using flow cytometry, a technology for counting and sifting cells. The researchers receive their results, but HIMC also stockpiles the data. Within a year or two, says Maecker, the center should have enough measurements from multiple studies to start nailing down



Back to basics. Stanford's Mark Davis wants immunologists to pay more attention to human immunity.

what's normal for the immune system.

The U.S. National Heart, Lung and Blood Institute in Bethesda, Maryland, has established a similar facility, the Center for Human Immunology, Autoimmunity and Inflammation. The center, says Director Neal Young, provides access to technology, such as the latest flow-cytometry and gene-sequencing machines, that researchers at the National Institutes of Health (NIH) might not be able to afford or have the expertise to use. Many of these studies—a current one tracks the effects of the H1N1 flu vaccine on 200 NIH employees—will be "just looking," Young says, and will accrue large amounts of basic immune data, which the center plans to make public.

Projects like these are a start, says Davis, but a concerted effort is needed. Large-scale projects are not only cheaper because of economies of scale, but they will also use standard procedures that yield comparable results. Moreover, squeezing the most information from blood samples will require an assortment of experts—from clinicians to bioinformatics virtuosos—who aren't available to every lab.

Banchereau is supportive of Davis's call. So is Ralph Steinman of Rockefeller University in New York City, who suggests that such a project could benefit one of his areas of interest: vaccines. "The truth is that to push vaccine science—say, for HIV or cancer—will require a major effort in human immunology."

Davis's push for more basic research on human immunity has also impressed people who control the scientific purse strings. He's received grants for such work from the Howard Hughes Medical Institute and the Bill and Melinda Gates Foundation. And last year the U.S. National Institute of Allergy and Infectious Diseases announced that it would spend \$100 million over five years on "human immune profiling research centers" that will track how our immune system responds to jolts such as vaccination and infection. The first grants are due to be awarded in May.

A human immunology project would require more research like this—and more money. Although Davis hasn't submitted a formal proposal, he suspects that the bill would be in the hundreds of millions of dollars, much less than the \$4.3 billion (in today's dollars) that the U.S. government spent on the Human Genome Project. Even some of the biggest fans of Davis's idea wonder, however, if such a project is affordable given the current economic climate. But unless we attempt to understand how our own immune system works, "we won't realize the health benefits of immunology," Davis says. "It's not a sustainable strategy to stay focused on mice."

—MITCH LESLIE



ECOSYSTEM MANAGEMENT

Science Meets Politics Off California's Coast

Protecting marine resources involves a mix of cutting-edge science and political compromise, as California officials have learned

Just offshore from San Diego, California, the largest kelp forest on the West Coast shelters rockfish, sculpin, and many other species. Anglers and sea urchin divers have plied those waters for decades, but not for much longer. The Department of Fish and Game is drafting regulations that would put 18 square kilometers off-limits to any fishing or harvesting. As a result, fish, lobsters, and urchins should start getting larger and more abundant, says Edward Parnell, a marine ecologist with Scripps Institution of Oceanography in San Diego. "They're going to fulfill their more historical ecological role," he predicts.

The reserve is just one of 48 marine protected areas (MPAs) designated by a new plan, approved in December, that would safeguard 15% of the state waters off Southern California. Under an ambitious \$38 million program that began in 2004, the state has already protected an even larger fraction of the Central Coast. Next up is the far northern reach of the coast. When the process is complete, California will have set aside more of its waters as no-take reserves than any other state, and perhaps as much as any country save Australia. "We've created an historic advance in marine conservation," says Gregory Helms of the advocacy group Ocean Conservancy in Santa Barbara, California. Other countries are beginning to emulate the approach.

But as the California experiment has shown, it's not easy. The process was authorized by a 1999 state law, the Marine Life Protection Act (MLPA), that called for a network

of protected areas to rebuild and protect marine ecosystems. From the outset, scientists were heavily involved in figuring out the most effective locations for MPAs. After their initial efforts met with heated opposition, it became clear that the needs of the fishers had to be accommodated, too. So a political dance ensued and compromises were struck—especially in Southern California, where controversy has been most intense. The recently approved plan there is "not predicated on making the best network but on making the best possible bargain," says Joel Greenberg of the Recreational Fishing Alliance.

Not surprisingly, fishers are still worried about the economic impacts, and conservationists have been upset about the failure to protect the very best habitat in Southern California. But the disagreement among these warring camps has been eased by a new bioeconomic computer model for designing MPAs in a way that is most likely to maximize biological benefits while minimizing economic pain. It should be a useful tool, its creators say, for other nations that are setting out to protect their coastal waters.

Networking

During the 1980s, California's waters were generating worrisome headlines. Populations of rockfish and other fisheries were crashing, raising larger concerns about the condition of marine ecosystems. The state already had more than 100 MPAs before the passage of MLPA, but they were not carefully planned, says Satie Airamé of the MLPA Initiative, the public-private partner-

Battleground. Productive kelp forests are prized by ecologists, fishers, and others.

ship that is running the process. The new law called for rigorous science to design MPAs that would function as a network to conserve marine life more effectively.

The first attempt to implement the act failed miserably. In 2001, the Department of Fish and Game asked a team of eight scientists to create a draft map of proposed MPAs. When it was released for public comment, communities near the proposed reserves howled. "If you showed up wearing a tie or looking like a scientist, guys would throw shrimp at you," recalls Christopher Weible, a public policy expert at the University of Colorado, Denver, who says it can take a decade to develop trust and work out details in designing an MPA. By the end of the year, the plan was shelved. A second attempt the next year included stakeholders but ran out of money.

The process didn't really get going until newly elected governor Arnold Schwarzenegger added his considerable political momentum to marine conservation. Five foundations pledged \$20 million to boost the state's funding, and the MLPA Initiative resumed work. The staff, drawn from various state agencies and nonprofit groups, divided the coast into four regions plus San Francisco Bay—a good move to reduce complexity, Weible says. The idea was to start in the Central Coast, learn from the experience, and then proceed to the next region.

First, however, a team of scientists created guidelines for the size and spacing of the protected areas. In a conceptual change from past efforts, the guidelines aimed to ensure that larvae could float from one MPA to another, based on estimates of how far they can travel. This would allow the populations in individual reserves to boost each other, creating a network of protected areas more resilient than if they were isolated. Another goal was to make sure that each kind of habitat was protected by more than one MPA. To meet these goals, 18% to 20% of state waters would need to be protected, says marine ecologist Steven Gaines of the University of California, Santa Barbara (UCSB), who helped craft the guidelines.

Next, the stakeholders got their turn. Anyone with an interest in coastal waters could participate: commercial and recreational fishing groups, for example, as well as marina operators and preservationists. Beginning in 2005, more than 50 representatives were charged to propose a set of networks in the Central Coast region. The proposals were

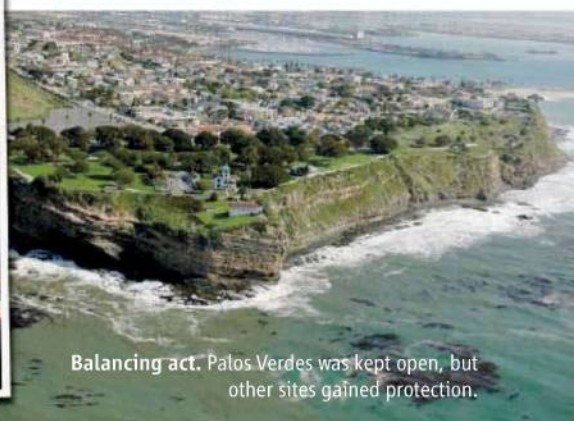
reviewed by the science advisory team to check whether the networks met the guidelines for size, spacing, and habitat coverage. Then a Blue Ribbon Task Force, appointed by Schwarzenegger, forwarded the plans, along with a preferred alternative, to state officials for a final decision.

During the meetings on the Central Coast reserves, fishing groups had complained that the scientific advisory team didn't have an adequate number of fisheries scientists. So for the next section of coast, to the north, the MLPA Initiative recruited Ray Hilborn, a fisheries scientist at the University of Washington, Seattle, and others. Once involved, Hilborn expressed several concerns. Most fundamentally, he argued that there is no need to locate MPAs so that larvae can travel from one MPA to another, because plenty of larvae exist in between them. By establishing what he views as arbitrary guidelines based on larvae dispersal, the science advisory team had "manipulated the system to get a certain amount of state [waters] put in reserves," he claims. Other members of the science advisory team disagreed, and the guidelines remained.

To help move past the debate, Christopher Costello, a UCSB economist on the science advisory team, and others wanted to focus on evaluating proposed MPA networks better. Costello, Hilborn, and Carl Walters of the University of British Columbia, Vancouver, in Canada developed a bioeconomic model that incorporates the habitat, ocean currents, and the biology of species, as well as fishing patterns. Another group, from UC Davis and headed by fisheries scientist Louis Botsford, adapted another model for the same purpose.

Rather than just pointing to guidelines, the models allowed the science advisory team to give much more explicit advice to stakeholders about the relative impact on conservation and fishing of placing MPAs in various locations. They could also project the costs and benefits of making a particular reserve bigger or smaller. The model was "worth its weight in gold," says Helms of the Ocean Conservancy.

On the South Coast, the model helped negotiate the toughest point of contention: the Palos Verdes Peninsula just west of Long Beach. With the best rocky habitat in much of Southern California, the site was a top priority



Balancing act. Palos Verdes was kept open, but other sites gained protection.

for preservationists. But creating a no-take zone there would cause big economic losses for charter fishing operations and restrict access by other users. When the political appointees on the Blue Ribbon Task Force decided not to protect the site, the model pointed to a second-best approach to conservation, emphasizing the need for MPAs around Malibu and Catalina Island, which had similar habitats. "The clearer you can be about what is at risk and why you're opting for one

dock," he says, and ignores the larger effects on the community, such as businesses that cater to fishers.

Preliminary research raises the possibility of a better outcome. The bioeconomic model suggests that MPAs, if designed properly and coordinated with fishery management, could boost fishery profits. Costello and colleagues took data from the South Coast region and compared the outcome of fishery management with and without MPAs. The value of the fishery doubled for kelp rockfish, for example, when MPAs were located in places that produce a lot of larvae, they reported online 22 February in the *Proceedings of the National Academy of Sciences*. Hilborn and others are skeptical. "I think they're a long way from convincing people in the fishery management community," Hilborn says.

Another unknown is whether the network of connected MPAs will perform better than a set of isolated protected areas would have. Marine ecologist Mark Carr of UC Santa Cruz says the only way to answer that ques-



Connect the dots. Dozens of new reserves were located to boost the dispersal of organisms while attempting to minimize economic pain to human users.

solution over another, the more acceptable the decision is," says Margaret Caldwell of Stanford University Law School in Palo Alto, California, a member of the task force.

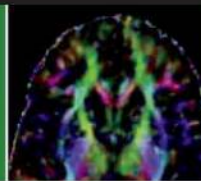
Win-win?

The model hasn't eliminated controversy by a long stretch. Bob Bertelli, president of the California Sea Urchin Commission, thinks that the Costello model—as well as a social science survey commissioned by the MLPA Initiative—underestimates the economic impact of designating MPAs, noting that they only estimate the lost value from smaller catches. "All the information stops right at the

solution will be to use computer simulations, and it could take 10 years to get enough data. To help figure all this out, the California Ocean Science Trust, a nonprofit foundation, has pledged \$16 million for monitoring the MPAs.

Even before the California process is done and its effects tallied, other countries are taking note. "It's been watched globally," says Jason Hall-Spencer of the University of Plymouth in the United Kingdom, a member of a scientific advisory panel now drafting ecological guidelines for a set of U.K. protected areas. "We're adopting many of the ideas."

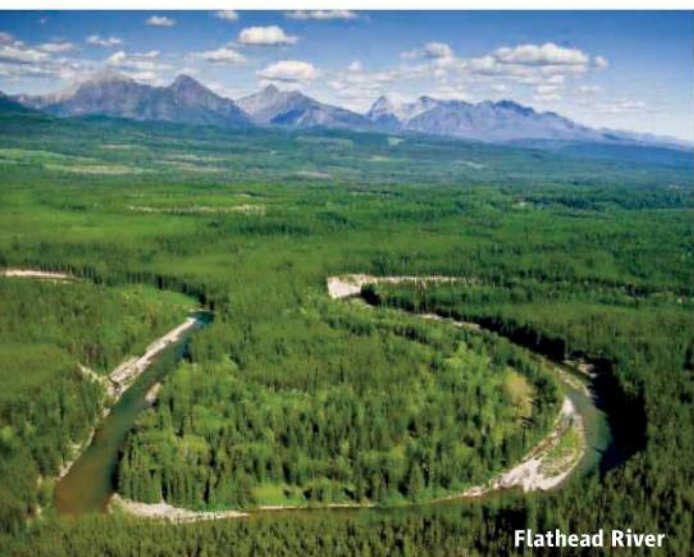
—ERIK STOKSTAD



LETTERS

edited by Jennifer Sills

Compelling Science Saves a River Valley



Flathead River

these plans from coming to fruition, included three elements: a careful scientific analysis, a fact-finding mission that respected the scientific input, and a productive diplomatic relationship that resulted in policy changes.

First, the United States responded by developing a science team to conduct a comprehensive environmental assessment of water quality, aquatic food webs, habitat, native fish, and wildlife. The science was compelling. A comparison between data collected from the Flathead and from the neighboring Elk River, the site of more than 50 years of open-pit coal mining, showed that waters of the Elk basin were significantly more polluted than those of the Flathead: Elk basin had more than 1000 times the nitrates, 100 times the sulfates, and 10 times the selenium concentrations. Similarly, aquatic food webs in coal mine-affected waters lost biodiversity as many pollution-sensitive species disappeared (2). In contrast, the pristine water and aquatic habitats in the Flathead support migratory populations of endangered species such as bull trout and nonhybridized westslope cutthroat trout (3).

In September 2009, a joint United Nations Educational, Scientific, and Cultural Organization (UNESCO)/International Union for Conservation of Nature fact-finding mission visited the Flathead in Montana and British Columbia to investigate whether the proposed mining was a threat to Waterton-Glacier International Peace Park, a UNESCO-designated World Heritage Site and Biosphere Reserve. The UNESCO mission listened carefully to the scientists' results. Their report concluded that mining in the Flathead would be "incompatible" with Waterton-Glacier as a World Heritage Site (4).

Finally, diplomacy at the state/provincial level and at the federal level between the United States and Canada was developed through personal relationships and mutual interest. Policy-makers in both countries respected the scientific and fact-finding analyses. As a result, on 18 February 2010, Premier Gordon Campbell of British Columbia and Governor Brian Schweitzer of Montana signed an accord to prohibit coal mining, coal-bed methane extraction,

and gas and oil exploration and development in the transboundary North Fork of the Flathead River Basin.

Throughout this process, scientific results played a central role in providing the backbone for resolute policy and the case for relentless political pressure. This healthy precedent will allow science to continue to inform policy as Canadian and U.S. officials work together to develop a natural-resource policy that protects this remarkable, shared ecosystem. We believe that this case will stand as an international model in which the natural goods and services provided by a World Heritage Site and Biosphere Reserve were ultimately valued over the commodities of natural-resource extraction.

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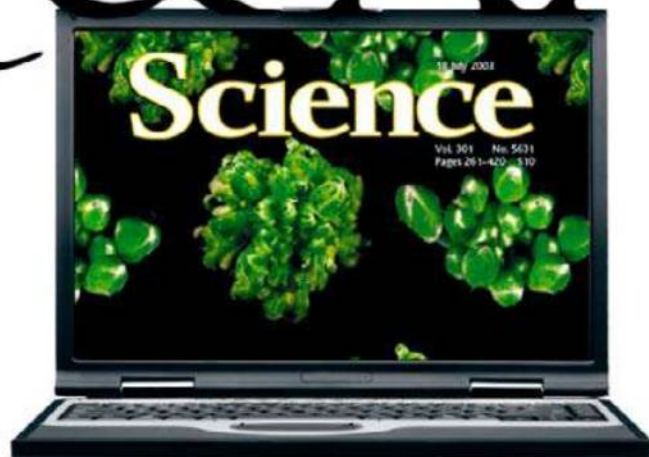
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Asian Test-Score Culture Thwarts Creativity

ASIA HAS BEEN HAILED AS THE NEXT GLOBAL science player as fast-growing Asian economies invest heavily in science and technology to drive further growth. However, Asian science will continue lagging behind the West because the Asian education system does not nurture

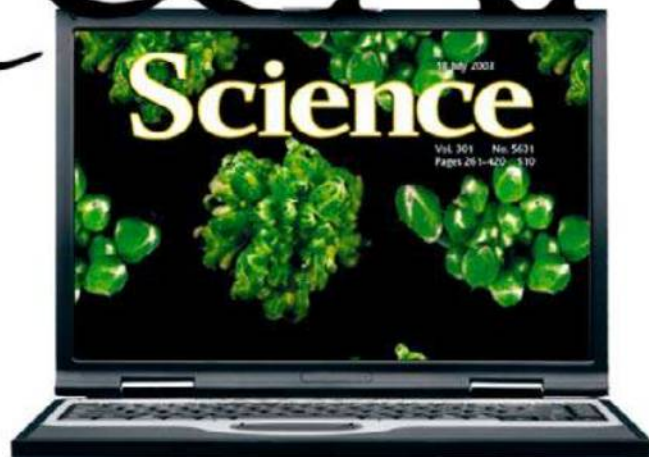
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Shedding light
on dark matter?

1582

SPORE prize
essay

1589

the creativity and thinking skills required in successful scientists.

In Asia, high scores determine the future career of the students as well as which schools get more funding. Teachers, under pressure to maintain their school's scoring record, teach to the test and organize extra classes for exam drills. Parents ferry children as young as first grade to centers for private tuition and exam preparation after school and on weekends. In Singa-

pore, a 2008 poll found 97 out of 100 students enrolled in private tuition (1). Last year, the test preparation industry was worth \$16.3 billion—36% of the public education expenditure—in South Korea (2), a country where, on college entrance exam day, parents pack churches and temples to pray and flights are rescheduled to reduce noise (3). Amidst pressure from long school days and

heavy homework, the Asian student's most intellectually demanding work is memorizing facts for regurgitation. The product of this educational culture is deficient in the inquiry, investigation, and reasoning skills needed for scientific discovery.

My experience with aspiring local graduate students has been that while motivation and work rate is usually high, they are weak at seeing connections in the published literature, extrapolating ideas, and generating hypotheses. The fact that they are top test-takers suggests that the Asian education system does not foster scientific talent.

East Asians have been described as strong in absorbing existing knowledge and adapting existing technology, but weak in making original contributions to basic science (4). A radical transformation of the educational culture must happen before homegrown Asian science can challenge Western technological dominance.

WILLIAM K. LIM

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LIFE IN SCIENCE

Anaconda!

For years a 25-foot-long reticulated python, known as Samantha, highlighted my herpetology class's visits to the Bronx Zoo. What would it be like, I'd wonder, to live in a world of mainly chemical, tactile, and infrared sensations, with one's head so far from one's tail? How would her presence affect other animals?

Despite decades of studying snakes, I lacked field experience with the few giant species until one evening in 2007, when—with my wife and her Brazilian research collaborators—I entered a Mato Grosso swamp to collect treefrogs. Our headlamps sliced back and forth through the knee-deep wetland, its vegetation glistening under a poignantly full moon.

Suddenly, I noticed staggered, dark coffee saucers winding past my left boot, like black leaves twisting in an olive current, and after a few dissonant seconds my brain flashed in recognition: a green anaconda! As her tail came into view, I grabbed with one hand, whereupon she jerked loose and reappeared several feet away. I took a few steps and lightly grasped the massive torso, worried that restraint would provoke constriction. As the enormous creature slid through my hands, I seized the 3-inch-thick tail and pulled up hard.

The next few seconds were chaotic. Her body glinted like a submarine parting the moonlit waters, then arched sideways, and I wondered if big toothy jaws were swinging our way. Instead the snake—which must have been 15 to 20 feet long—strained forward, sliding against my left hand, and I struggled backward clutching the tail in my right. Her skin was slick and tough, neither cold nor slimy. She moved with muscular confidence, as if I were a momentary distraction.

Inexorably the huge snake pulled free. But thanks to that night, I more viscerally appreciate how she could appear as benign as leaves and stones on a pond bottom. I now know why a deer

or a monkey lingering at the water's edge might not detect an anaconda's stealthy presence. There are no sounds as she approaches, no scented warnings from upwind, and sinuous ripples in the swamp grass could just as easily be a storm's breezy prelude. I now understand why complacency doesn't bode well in the home of giant serpents.

HARRY W. GREENE

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EDITOR'S NOTE

This is an occasional feature highlighting some of the day-to-day humorous realities that face our readers. Can you top this? Submit your best stories at www.submit2science.org.



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CORRECTIONS AND CLARIFICATIONS

News Focus: "The tangled roots of agriculture" by M. Balter (22 January, p. 404). The first paragraph refers to "hunter-gatherers in what is now Israel, Jordan, Syria, and Lebanon." This list, although not comprehensive, also could have included the Palestinian territories, as a few sites are within those borders.

Reports: "Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species" by J. H. Werren *et al.* (15 January, p. 343). The author list on page 343 should read as follows: John H. Werren, Stephen Richards, Christopher A. Desjardins, Oliver Niehuis, Jürgen Gadau, John K. Colbourne, and The *Nasonia* Genome Working Group. The list has been corrected on the online HTML page.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

SOCIAL SCIENCES

History as a Laboratory

James Mahoney

In the conclusion to his Pulitzer Prize-winning *Guns, Germs, and Steel*, Jared Diamond argued that historical researchers face challenges in establishing cause-effect relations not unlike scholars in fields such as astronomy, evolutionary biology, and geology (1). In *Natural Experiments of History*, Diamond, coeditor James Robinson, and their contributors pick up where the earlier book left off by addressing the specific methodological issues involved in making valid causal inferences about the past. Although the new collection of essays is mainly by and for social scientists, Diamond and Robinson clearly also want it to speak to natural scientists and historians. To the natural scientists, their message is simple: please don't dismiss the possibility of studying historical phenomena scientifically. When speaking to the historians, their advice pushes in the other direction: please overcome any reluctance you may have about embracing scientific methods as the basis for making inferences.

Historical analysts cannot, of course, test their ideas by running controlled experiments. They cannot randomly assign cases to treatment and control groups. But they can sometimes make a credible claim that the assignment of cases to different groups is "as if" random. The label "natural experiment" (or "quasi-experiment") is often reserved for those studies in which this assumption seems especially plausible (2, 3). However, Diamond and Robinson choose to include under its rubric a broader range of studies in which investigators use statistical methods to adjust for imbalances across treatment and control groups.

One of the joys of reading social science history is precisely probing the credibility of the as if random assumption embedded in an explanation of an intriguing puzzle about the past. The assumption seems reasonably plausible in Abhijit Banerjee and Lakshmi Iyer's chapter about the consequences of colonial land systems for the creation of public goods across different regions of India. They find that areas where the British empowered big landlords have far fewer schools and roads today compared with places where landlord-based systems were not imposed. Their claim

that this association is a causal relationship seems convincing because "the land revenue systems put into place in different parts of India by British colonial administrators ... had nothing to do with any special feature of the areas where they were imposed." Unfortunately, as the authors note, there were some systematic differences related to geography and population density between areas that received landlord-based systems and those that did not. Although they worked hard to control for these differences, the absence of true randomization still raises the concern that something other than colonial policy might explain the current variation in public goods.

Some authors acknowledge that the as if random assumption is not really plausible but nevertheless try to show that valid causal inference is still possible. For example, Nathan Nunn poses a fascinating question: Did the trans-Atlantic slave trade contribute to the underdevelopment of African societies? Using multivariate regression analysis, he concludes that the answer is "yes"—African nations exhibit a strong negative relationship between the number of slaves exported (normalized by land area) and their contemporary wealth. A complicating issue is that slaves were taken primarily from more densely populated regions. Nunn interprets that fact as favoring his answer: Densely populated areas tended to be more economically developed, and thus slaves came from regions that were previously richer but that are now poorer. An alternative interpretation is that slaves were extracted mainly from regions with entrenched precapitalist institutions. I worry that such prior divergences, not differences in slave exportation, explain the variation in contemporary levels of development (4).

Reading through the book, one can't help but notice a possible trade-off between the plausibility of the as if random assumption and the number of cases analyzed. As Dia-

mond notes, classic natural experiments in the social sciences usually encompass only a small number of cases, as when a border is arbitrarily drawn between two regions (e.g., the 1945 separation of East and West Germany). These studies often can make a good claim for the random assignment of cases into different sets according to some variable of interest (for example, public policies). But with only a small number of cases, it is hard to know whether the treatment of interest (as opposed to other antecedent variation among the cases) actually

caused subsequent differences. For example, in his chapter on inter-island and intra-island comparisons, Diamond asks why Haiti is now so much poorer than the Dominican Republic. One component of his answer involves the creation of the border that divides Hispaniola into the two countries. The west was colonized by France; the east by Spain. That difference

Natural Experiments of History

Jared Diamond and James A. Robinson, Eds.

Harvard University Press, Cambridge, MA, 2010. 286 pp.
\$29.95, £22.95, €27.
ISBN 9780674035577.



Border effects? Diamond compares the historical trajectories of Haiti (to the left) and the Dominican Republic (to the right).

had huge ramifications, including leading to a Haitian population composed mainly of slaves, while the Dominican Republic had relatively few slaves. Yet these two countries had other prior differences. Most important, as Diamond notes, the Haitian side of the island is drier and steeper, with less fertile soils. Consequently, I remain uncertain whether it is these initial geographic differences or the treatment of interest (i.e., the creation of the border) that holds the key to explaining the puzzle of Hispaniola.

Scholars across various disciplines may react differently to this book. Historically oriented social scientists like me will delight in reading the substantive chapters, which nearly uniformly raise fascinating ques-

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tions and represent high-quality scholarship. My guess is that most natural scientists will be sympathetic to the editors' mission and appreciate the seriousness with which the authors work to mimic an experimental logic using observational data. As for those historians who devote their careers to studying particular events, one can only hope that they will hear the editors' concluding words: "Yes, if you study an event for a long time, that does give you one type of advantage. But you gain a different type of advantage by taking a fresh look at an event, and by applying to it the experience and insights that you have gained by studying other events."

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4. Slave exportation may well explain why sub-Saharan Africa as a whole is especially poor. But Nunn's research is not directed at testing that hypothesis, which would require cross-continent comparisons.

10.1126/science.1187684

ENGINEERING

For Solutions That Will Work

David E. Nye

In *The Essential Engineer*, Henry Petroski argues that we need engineers if we are to find ways to solve such problems as generating renewable electricity, supplying clean water, maintaining reliable communications, and deflecting an asteroid before it hits Earth. The opening chapters examine the vexing differences, real and perceived, between scientists and engineers. All too often, the flawless rocket launch is described as a scientific achievement, whereas delays or failures are labeled engineering problems. Likewise, the more successful an engineer becomes, the more likely newspapers will call him or her a scientist. Petroski (an engineer and historian at Duke University) feels that engineers often do not get the credit they deserve. Certainly there is no Nobel Prize in

The Essential Engineer
Why Science Alone Will
Not Solve Our Global
Problems

by Henry Petroski

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286 pp. \$26.95, C\$33, £17.89.
ISBN 9780307272454.

engineering. All the same, they have not necessarily been subordinate to scientists. Engineers have often served as chief executives of major corporations (e.g., Alfred Sloan at General Motors), and an engineer, Leslie Groves, was in charge of the Manhattan Project. Furthermore, if popular culture often presents us with the figure of the "mad scientist" there is no comparable figure of a "mad engineer." Jokes suggest that scientists are perceived as eccentric, at times even flamboyant, while one can spot "an extroverted engineer" because "she is the one who looks at your shoes during a conversation."

Many engineers, including my father, have been irked that in the 20th century scientists get most of the glory, although engineers are essential to launching rockets, building atomic accelerators, and making marketable wind turbines. In contrast, during the 19th century Americans were more enamored of inventors and engineers, from Eli Whitney to Thomas Edison to the Roebling family who built Brooklyn Bridge. Such engineers were not merely finding practical uses for scientific theory. During much of human history, builders and tinkers have created things that worked before anyone could explain them through scientific laws. Fortunately, practical people did not wait for Newton to develop a theory for gravity before putting it to use. Some Victorian scientists claimed that the electrical current could not be subdivided, but Edison ignored their advice and developed the infrastructure for electrical generation and transmission. Later, Charles Steinmetz and others developed mathematical models that helped utilities to understand more precisely what electricity was doing in their systems.

Much of this readable book focuses on questions that have been in the news during the past decade, such as how to deal with global warming, which alternative energy technologies make economic sense, and how to prepare for disasters such as hurricanes and earthquakes. In each case, Petroski offers a succinct overview of the problems and potential solutions, providing an evenhanded assessment. For example, when discussing energy he does not champion geothermal, wind, solar, or nuclear power but explains the advantages and drawbacks of each. He also notes the irony of many well-



"From the creative mind" of engineers. John Fowler and Benjamin Baker's Forth Railway Bridge, Scotland (completed 1890).

meaning reforms: Highly polluting cars have been outlawed from American roads only to be exported and remain in use elsewhere. A Massachusetts power plant was decommissioned and, at a cost of \$22 million, taken apart and shipped to Guatemala, where it pollutes the atmosphere at least as much as it did before. Petroski also argues that from an engineering perspective, some scientific "solutions" (such as the proposal to spend trillions of dollars on "artificial trees" that can remove carbon from the atmosphere) appear wildly impractical. And as readers of the author's *To Engineer Is Human* (1) might expect, the book discusses some spectacular design failures: the new Denver airport's malfunctioning baggage handling system, the persistent blackouts that have plagued the U.S. electrical grid, and the notoriously uneven performance of the air conditioning system at the United Nations. Petroski also notes the ironies of success. For example, the excellent safety record of the airline industry has made it difficult to further improve safety, because there now are so few new accidents to analyze.

Although individual chapters can be read separately, they form part of a larger argument that engineers can build the needed bridges across the gap that C. P. Snow identified between the "two cultures" of the humanities and the sciences. To the extent that this divide has been closed in recent decades, Petroski's dozen books surely deserve considerable credit. Characteristically, the closing pages of *The Essential Engineer* call on scientists and engineers "to come together and work as the team that they naturally should be."

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10.1126/science.1188527

The Future of Psychiatric Research: Genomes and Neural Circuits

Huda Akil,^{1*} Sydney Brenner,² Eric Kandel,³ Kenneth S. Kendler,⁴ Mary-Claire King,⁵ Edward Scolnick,⁶ James D. Watson,⁷ Huda Y. Zoghbi⁸

The burden of neuropsychiatric illnesses is enormous. These conditions, which include schizophrenia, mood disorders, and autism, affect thought, emotions, and a person's very sense of self. Together, they are the leading cause of disability in North America and Europe and constitute 40% of all years lost to disability. In the United States, the cost in lost earnings due to psychiatric disease is estimated conservatively to be \$200 billion per year (1). The burden to individuals, families, and society is all the more tragic because these illnesses typically begin early in life, are lifelong, and damage the affected individuals' self-perception, productivity, and ability to relate to others.

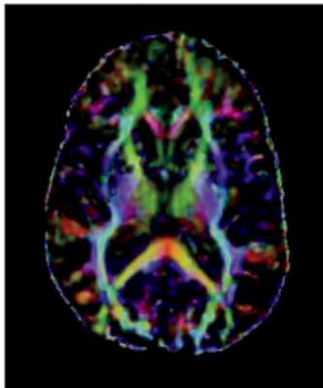
Unfortunately, there have been no major breakthroughs in the treatment of schizophrenia in the last 50 years and no major breakthroughs in the treatment of depression in the last 20 years (2). Over the last few decades, drug treatments have emerged that help a subset of these patients (3), but a sizable proportion are resistant to all currently available treatments. This heterogeneity points to the complexity of the underlying biology and underscores the urgent need for a more sophisticated understanding of the causes of these illnesses.

Psychiatric disorders present a unique challenge, even relative to other brain disorders, such as Alzheimer's, Huntington's, or Parkinson's diseases, because for psychiatric disorders we know much less about

underlying genetic, molecular, and cellular causes or even the primary anatomical sites of the brain defects. This frustrating lack of progress requires us to confront the complexity of the brain, especially in the context of higher-order functions, such as cognition and mood. This calls for a new perspective and a combination of novel tools and analytical methods.

Disruption of Neural Circuits

Illnesses such as schizophrenia, autism, and mood disorders are likely the result of disruptions of neural circuits, the functional ensembles of brain cells that mediate



Diffusion tensor imaging (DTI) of a normal adult brain depicting white matter pathways (15 directions, $b = 800 \text{ mm}^2/\text{s}$). This tool can be used to assess changes in neural connectivity associated with brain disorders.

thought, feelings, and behavior. A defect in the development, anatomical structure, functional integration, or dynamics of such a circuit can lead to a constellation of symptoms. Given the complexity of

neural circuits, there are many possible ways to disrupt them (4). Thousands of genes are involved in regulating neural development and function. It is not surprising, therefore, that disturbances in the structure and function of one or several of these genes can lead to broad and complex neuropsychiatric phenotypes. This complexity explains the high prevalence of neuropsychiatric disorders. It also indicates that many genetic mutations, epigenetic changes, and other cellular and morphological brain lesions can converge on disturbing a given brain circuit and result in shared clinical manifestations (e.g., delusions and hallucinations) that lead to the same clinical diagnosis (e.g., schizophrenia). Thus, starting from a diagnosis and searching broadly for genetic causes that are commonly shared across all affected individuals is not likely to succeed, because a great deal of biological heterogeneity lies at

Integrating the tools of genomics and neural science is needed to reveal causes of neuropsychiatric illnesses and to suggest new strategies for treatment and prevention.

the basis of circuit dysfunction.

This does not mean, however, that these diseases are not genetically based and transmitted, nor does it suggest that the search for genetic causes will be fruitless. Indeed, ample evidence demonstrates the heritability of these disorders. For example, there is much greater concordance of diseased states in identical twins versus fraternal twins. In autism, in as many as 80% of families of identical twins, both display autistic features if one is affected (5). Concordance of schizophrenia is estimated to be ~50% in identical twins, as opposed to ~5 to 10% in fraternal twins (6, 7). In some persons, nongenetic factors—such as intrauterine infection, malnutrition, or stress—may also be required to trigger the illness (8). Because of these complexities, most of the genes involved

in the major psychiatric illnesses have not yet been identified, and animal models for them are limited.

Recent studies indicate that for many patients, psychiatric illnesses are due to genetic vulnerabilities that are shared by affected members of a given family, but vary across families, such that a given family has a unique, or “private,” mutational profile (9). It is therefore critical to focus on approaches that can detect private mutations and will take into account the genetic and neurobiological heterogeneity of psychiatric disorders.

Genomics and Circuit Analysis

What is the best strategy for unraveling the biological causes of psychiatric illnesses? We suggest that their solution will require the integration of two general approaches that have matured dramatically in the last 3 years: Genomics and Circuit Analysis.

Genomics is the combination of large-scale sequencing with systematic computational analysis of genomes. In the last 2 years, sequencing the human genome has become significantly faster and much less expensive. This makes it feasible to sequence the complete genome of many afflicted individuals to discover the genetic bases of these disorders within subjects and families. It is no

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longer necessary to target specific genes or chromosomal regions based on preconceived notions about the nature of the genetic defect. This approach has already proven useful in the analysis of X-linked mental retardation (10). New insight into the complex genetic basis of psychiatric disease comes from recent analysis of gene copy number variants present in patients with autism, schizophrenia, and bipolar disorder (11). Some of these mutations are absent from either parent's DNA, which suggests that they are de novo mutations. Many deleted or amplified DNA segments contain genes needed primarily for the functioning of nerve cells, not for cells in other tissues. Most of the neural genes thus far identified as mutant have been observed only once, which suggests that the total number of genes whose disruption can result in the severe mental illnesses is at least several hundred. Mutations in many different genes have the potential to cause the neural circuit malfunction of psychiatric illness. It is therefore critical that computational biologists be engaged in analyzing the results of this sequencing effort in order to ascertain the contribution of the observed variants or allelic combinations to the disease in the general population.

Circuit analysis is the study of the structure, function, and dysregulation of relevant neural circuits. We are now beginning to identify the possible locations of aberrant circuitry in some diseases, including depression, which is associated with hyperactivity in the subgenual cingulate region (Brodmann area 25) of the prefrontal cortex (12); anxiety states, where there is hyperactivity in the amygdala (13); and obsessive-compulsive disorder, where there is an abnormality in the striatum (14). We need biological markers for the disruptions in neural circuitry involved in mental disorders in order to fully elucidate the anatomical basis of each illness, to provide more objective diagnoses, and to follow responses to treatment.

Fortunately, there have been parallel advances in the development of a variety of powerful new methods for selectively analyzing neural circuitry, which allow the delineation of the brain-wide neuroanatomical connectivity patterns in normal experimental animals, in animal models of psychiatric illnesses, and in people. One example is the introduction of noninvasive ways to selectively activate or shut off specific neurons in a given circuit of a behaving animal using optical methods (15). Also effective for selectively turning neurons on or off are ligand-gated protein variants with custom-

ized binding sites, such as the *Drosophila* allosteric receptor and the G_i protein-coupled receptor (16). Neither of these techniques can be readily used in humans. However, other very recent technical advances include methods for tracing neural connections in humans using diffusion tensor imaging (17) (see the figure on page 1580); approaches to the analysis of gene expression and epigenetic modifications in animal and human brains (18, 19); and tools for studying cellular and circuit dynamics in the brain, including multiphoton imaging and calcium imaging coupled to creative electrophysiological strategies (20).

Because genes responsible for Huntington's disease, Rett syndrome, Fragile X, and early-onset Alzheimer's disease have been discovered, it has been possible to express these genes in mouse models and to learn a great deal about the mechanisms of pathogenesis. As new genetic variants are discovered for psychiatric disorders, it will be possible to introduce these mutations into mouse models to simulate the human disorder, providing badly needed insights into the pathogenesis of these disorders. The impact of these mutant genes on circuit development, structure, function, and pathogenesis can now be studied using the innovative approaches described above. The convergence of findings from multiple animal models with the critical mutations will help to identify the central features of circuit dysregulation that are shared regardless of the initial genetic defect. It is these shared features that are likely to underlie the human disease that is being modeled. This understanding will also allow us to devise new treatment strategies that target either specific defects or their final common path and can reset circuit function in spite of the heterogeneity of the genetic causes of its malfunction.

An International Project

Now is the time to initiate an effort that relies on the combined power of these two critical sets of tools. One component of this effort would aim to provide the world of medical neuroscience, over the next decade, with the personal genomes of many patients who have been afflicted with severe psychiatric diseases, especially those with the clearest evidence for a genetic contribution, along with appropriate controls. The effort would begin by focusing on autism, schizophrenia, and bipolar disease, but would eventually include the study of other severe psychiatric disorders, especially major depression. For schizophrenia and bipolar disorder, thousands of DNA samples from carefully evaluated patients

and their families are already available and thousands more are being collected.

A parallel component of this effort is tackling the study of neural circuitry, in animal models and in human subjects, to allow functional insights into the way that these altered genes can disrupt circuit formation, function, and dynamics. The neuroscience technologies described above tend to exist in highly specialized settings. Significant intellectual, technical, and financial investments are needed to refine them, to establish them broadly, and to integrate them with each other and with genetic analysis, so as to provide the tool kits needed by the scientific community.

Soon the cost of sequencing and analyzing single personal genomes will be in the \$5000 to \$10,000 range. Assuming that the inherent complexity of neural circuitry disease demands that we need to consider 100,000 personal genomes, we would need only a billion dollars over the next 10 years to do so. A similar figure for tackling neural circuitry would allow functional insights into these genes and inform us about how these genes can malfunction and cause disease. Two hundred million dollars a year, the sum of monies that will be needed, is a very small price to pay to reduce or eliminate the awful misery and burden to society caused by mental illness.

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ASTRONOMY

Fishing for the Universe

Rafael F. Lang

It is a sobering fact that of all the matter in the universe, only 17% is made of particles we know (1). Little is actually known about the remaining 83%, but we do have a name for it—dark matter. On page 1619 of this issue, the Cryogenic Dark Matter Search (CDMS II) Collaboration (2) presents tantalizing results from their recent experimental run that may provide an answer soon to the question of what all that dark matter is made from.

A promising dark matter candidate is a massive particle (a few to a few thousand proton masses) left over from the Big Bang, a weakly interacting massive particle (WIMP). Because WIMPs may annihilate each other upon collision, emitting detectable radiation or other particles, satellite- and balloon-borne experiments can be used to search for particles that may be

produced in these processes, for instance, at the center of our galaxy. Although several experiments have measured signals in excess of that expected from astrophysical sources, e.g., (3), the challenge is to understand the backgrounds well enough to convincingly claim the discovery of a dark matter annihilation signal.

A more direct approach is the detection of dark matter particles interacting with ordinary matter in a laboratory experiment. Major technological advances have allowed us to build detectors with an unprecedented sensitivity to such rare events. Current detection levels limit the search to less than one single WIMP caught in the mass equivalent of a human body per week. However, in every one of us, more than 4000 atoms of potassium decay every second. The issue then is in distinguishing that one dark matter interaction from the vast background induced by abundant natural radioactivity.

In order to reduce the background from ambient radioactivity, the CDMS experi-

After analysis of a year-long detection experiment, resolution of the dark matter mystery may be near.

ment has been located in the Soudan Underground Laboratory in Minnesota, below more than 700 m of solid rock and behind a thick lead and polyethylene barrier. They use precision detectors made from ultrapure germanium crystals with a mass of 230 g each. For every particle interaction in the crystal, both an ionization signal and a phonon (heat) signal are read out. Electrons and gamma rays constitute the main remaining radioactive background, manifesting as recoiling electrons. In contrast, WIMPs scatter off target nuclei and can be distinguished from the background of electron recoils based on their lower ionization signal. This improves the signal-to-background ratio in the CDMS detectors by more than four orders of magnitude.

Taking data for more than a year, and performing a careful blind analysis of the recorded data, the CDMS II Collaboration had expected to observe about one event from background in the predefined signal area, but

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CREDIT: THOMAS TUCHAN

Out of the dark. The Milky Way is held together by dark matter. With ever more sensitive detectors, we might soon detect dark matter particles, clarifying the results presented by the CDMS II Collaboration (2).

instead they observed two. Although this result may not be exciting from a statistical point of view, it has been met with excitement in the field. For years, experiments have produced null results, providing only upper limits on cross sections of WIMP-nucleon interactions. The two detection events have raised hopes that more sensitive detectors may soon shed light on dark matter.

Because target materials can be made very pure, most radioactive backgrounds come from the surface of a detector, and instrumental effects are also stronger near detector edges. Continuing their two-decade long effort building improved detectors, the CDMS II Collaboration developed thicker germanium targets to improve the surface-to-volume ratio, and with more efficient phonon collection to better reject surface events. This increases the acceptance of these new detectors to dark matter events and allows them to take the next step in sensitivity that is required to clarify the result reported.

Meanwhile, other experiments use differ-

ent technologies, each with its own difficulties and advantages. Some use liquid noble gases as target material and read out the scintillation light that is created in a particle interaction. If electrons liberated in the interaction drift through the liquid and are measured as well, this allows for background discrimination similar to CDMS. The major advantage of these liquid target detectors is that they can be made large, hence decreasing the surface-to-volume ratio. The first of these to challenge the CDMS lead of the field was the XENON10 experiment (4). There, a large reduction of the radioactive background was achieved using only the innermost 5.4 kg from a 15-kg liquid xenon target. This year should see rapid progress, with at least three major liquid noble gas experiments expected to take and release data. Even ton-scale detectors will be feasible within a few years.

With all these highly sensitive detectors being or becoming operational, we can expect to learn soon whether or not CDMS observed dark matter and, with a bit of luck, what it

is made of. Further, we expect that, should dark matter be WIMPs, we will detect many of them within only a few years and be able to estimate of their mass. Then the real fun will begin, as WIMP astronomy will allow us to infer otherwise inaccessible information. For example, due to their feeble interactions, the energy distribution of WIMPs in our galaxy, the Milky Way (see the figure), preserves features more than a billion years old that arose during collisions with other galaxies (5). Hence, measuring this WIMP energy distribution with direct dark matter detectors would open the history book of our galaxy.

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ECOLOGY

A Green or a Prickly World?

Peter A. Hambäck

Is the world a green place where plant biomass abounds for herbivores to devour, or a prickly place where herbivores struggle to locate the few edible plant pieces? The answer to this question has crucial consequences for broad ecological and evolutionary questions. On page 1642 of this issue, Mooney *et al.* (1) show how evolutionary trade-offs among plant traits affect responses to herbivores and higher trophic levels.

If plant biomass is accessible but not consumed, herbivore numbers, and thus their consumption, may be regulated from the top by their natural enemies. If, in contrast, plant biomass is a nasty resource, then plant resistance to herbivory is central to understanding the greenness of the world. This dichotomy suggests a straightforward answer, but in nature, top-down forces interact in complicated ways with plant traits in determining the strength of species interactions. To address these issues, Mooney *et al.* have studied 16 related milkweed species. The results show that top-down effects from predator presence to plant biomass were determined not by plant resistance

or by interaction strengths between herbivores and their natural enemies, but rather by a trade-off between plant growth strategy and resistance to herbivory.

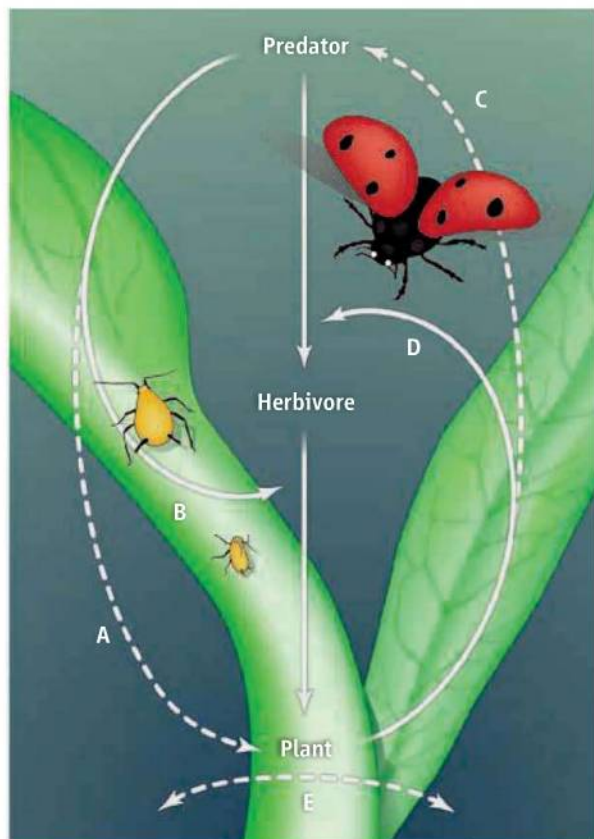
The evolution of species traits depends on the main selective factors determining fitness. Populations facing high risks of predation will evolve different traits than populations where food limitation and starvation is common. In nature, this dependence is not unidirectional (even though most experiments are so), and traits themselves likely feed back to affect the species interactions and the fitness landscape. When we try to understand the role of certain species interactions in a specific ecological situation, what we see is a product of the evolutionary history of involved species and the proximate ecological factors. The interplay between these temporal scales for indirect interactions is only beginning to be disentangled by work such as that of Mooney *et al.*

In many insect-dominated ecological systems, plants emit volatile compounds to attract predators. These emissions create feedbacks between predators and herbivores that affect plant biomass and fitness (2). Predators and parasitoids attacking herbivorous insects use the chemical cues provided

The evolution of plant traits shapes complex interactions with herbivores and their predators.

by plants at the time of herbivore attack to locate their prey. Mooney *et al.* (1) suggest that differences in volatile production—particularly of sesquiterpenes, which affect natural enemy attraction (3)—correlate with the effect of removing predators. Milkweed species that produced higher amounts of sesquiterpenes showed a larger difference in plant biomass with or without lady-bird beetles and other aphid predators, apparently because these species also differed in their tolerance to herbivory.

Most studies on the role of volatiles (the infochemical web) in plant-arthropod interactions have focused on mechanisms and chemical backgrounds, providing limited insights into the wider consequences for natural communities. Overlaying food webs with the infochemical web will increase our understanding of complex species interactions. In addition to the top-down effects on trophic cascades studied by Mooney *et al.*, this was illustrated in a recent study of two related herbivorous beetles, their respective host plants, and a shared parasitoid (4). Field surveys suggested that mortality rates of one beetle species (*Galerucella tenella*) were higher when the other species (*G. californiensis*) was pres-



A complex web. Food web interactions involve direct (solid lines) and indirect (hatched lines) effects. Predators and herbivores directly affect their resources through consumption, creating indirect effects of predators on plants (A). Predators also affect herbivore behavior and, indirectly, plant consumption (B) (8). Plants affect predator growth through effects on herbivore biomass and quality (C) (9) and predator search through damage-induced volatiles (D). Mooney *et al.* (1) suggest that trade-offs between plant growth strategy and resistance to herbivory moderate top-down effects (E).

to the scent of flowering meadowsweet (*Filipendula ulmaria*), host plant to *G. tenella*, and deposited more eggs in *G. tenella* larvae. As a result, attack was reduced and seed set increased on meadowsweet when growing together with the host plant of *G. calmarensis*, purple loosestrife (*Lythrum salicaria*). Herbivory from *G. tenella* can be very strong, defoliating the host plant, and selects for increases in defensive chemicals (5). This strong selection would presumably not occur in the presence of purple loosestrife.

Predators and parasitoids provide important ecosystem services to mankind and food production by reducing

pest densities. Estimates suggest that up to 15% of food production is lost to arthropod pests (6), and the global cost of biological

control is estimated at US\$417 billion/year (7). Yet, little is known about how to improve the conditions and efficiency of natural enemies in cropping systems. This development hinges on our detailed understanding of how complex interactions between plants, herbivores, and their predators determine the loss of plant tissue and the ability of plants to compensate for such losses. The integration of trophic dynamics and infochemical webs promises to provide deep additional insights to this end.

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ent in the neighborhood. Seemingly, *G. calmarensis* was the better host for parasitoids, but adult parasitoids were nevertheless attracted

CHEMISTRY

Less Costly Catalysts for Controlling Engine Emissions

James E. Parks II

Lowering the fuel consumption of transportation vehicles could decrease both emissions of greenhouse gases and our dependence on fossil fuels. One way to increase the fuel efficiency of internal combustion engines is to run them “lean,” in the presence of more air than needed to burn all of the fuel. It may seem strange that engines are usually designed to run with fuel and air at stoichiometric balance, or even fuel rich. However, the way emissions have been controlled with catalytic converters has required some unburned fuel in the exhaust, especially for controlling the nitrogen oxide pollutants NO and NO₂ (called

NO_x). On page 1624 of this issue, Kim *et al.* (1) report encouraging results for catalysts that can process NO_x in lean-burn engines. These perovskite oxide catalysts may help reduce or even eliminate the need for expensive and scarce platinum group metals (PGMs) in emission-control catalysts.

Commercially available vehicles with lean-burn technology have either diesel (2) or gasoline direct injection engines (3), and typically have additional costs of \$1000 to \$5000 compared with stoichiometric engine vehicles (4). A large part of the extra cost is due to engine modifications and exhaust catalysts that enable compliance with emission regulations.

Lean engines produce oxygen-rich exhaust, which prevents the reduction of NO_x

Perovskite oxide catalysts may be able to control emissions from more fuel-efficient internal combustion engines and replace expensive noble metals.

via the “three-way” catalyst commonly used for stoichiometric engines. The new combustion and catalyst technologies for lean engines must meet NO_x emission standards as well as those for carbon monoxide, hydrocarbons, and particulate matter (soot). For diesel engines, special exhaust filters have eliminated the unsightly plumes of soot emissions from diesel exhaust.

Two catalyst technologies for controlling NO_x emissions from lean-burn engines are urea-based selective catalytic reduction (SCR) and the lean NO_x trap (LNT), also known as the NO_x storage and reduction catalyst. Urea SCR’s main disadvantages are related to the requirement of onboard storage of the urea reductant and the need to heat the urea in cold weather. The issue of how

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to enforce resupply of the urea reductant by drivers must also be addressed.

In contrast, LNTs use engine fuel for NO_x reduction, but they require PGMs that add to the cost of lean engine vehicles (see the figure, panel A). Unlike the three-way catalysts, which reduce NO_x continuously, LNTs switch between two modes of operation. Most of the cycle is spent trapping NO_x during lean operation (see the figure, panel B). Periodically, control systems cause the engine to run fuel rich, and unburned fuel in the exhaust is used to liberate the stored NO_x and reduce it to N_2 (see the figure, panel C).

In selecting alternative catalysts, it has become important to understand the roles of PGMs in the various LNT processes (5–8). During lean operation, platinum (Pt) affects NO oxidation to NO_2 ; most of the NO_x is NO , but it must be oxidized to NO_2 for storage. During the transition to fuel-rich operation, Pt oxidizes hydrocarbons to fully eliminate oxygen from the exhaust, which must occur if NO_x is to be released from the trap. Finally, during the rich phase, Pt enables regeneration of the LNT for more NO_x storage by assisting in the NO_x release and reduction steps. Rhodium is also a common PGM in LNTs and is often added to promote NO_x reduction and hydrocarbon-processing reactions (9).

The amount of PGM added to LNTs depends on many engineering factors; levels of 3.5 g per liter of catalyst volume (10) are typical, and more than is used in the three-way catalyst. One key factor in LNT performance for passenger-car applications is NO_x reduc-

tion at the low exhaust temperatures generated by lean engines (diesel exhaust temperatures below 250°C are common at low engine power). At low temperatures, the effect of Pt on NO_x reduction performance is greater than at higher temperatures (11).

The catalysts developed by Kim *et al.* are based on the perovskite oxides, $\text{La}_{1-x}\text{Sr}_x\text{CoO}_3$ and $\text{La}_{1-x}\text{Sr}_x\text{MnO}_3$, where lanthanum (La) is a rare-earth cation, strontium (Sr) is an alkaline-earth cation, and cobalt (Co) and manganese (Mn) are transition-metal cations. In tests with simulated diesel exhaust, the $\text{La}_{1-x}\text{Sr}_x\text{MnO}_3$ catalyst achieved conversions of NO to NO_2 comparable to that of commercial Pt-based catalysts. These catalysts are less effective at hydrocarbon and carbon monoxide oxidation, and are also prone to deactivation by sulfur, a contaminant present in fuel. However, the oxidation activity of the catalyst could be improved in the presence of sulfur by adding palladium, a PGM. Palladium increases the performance of the perovskite-based LNT as well.

Volatility in PGM prices has been a problem in minimizing the cost of the technology. During the last decade, monthly average platinum market prices have ranged from about \$400 to \$2000 per troy ounce (12). With volatile and escalating prices, catalyst product costs vary dramatically during the course of a product development cycle. As a result, engineers are constantly chasing a moving cost target during development.

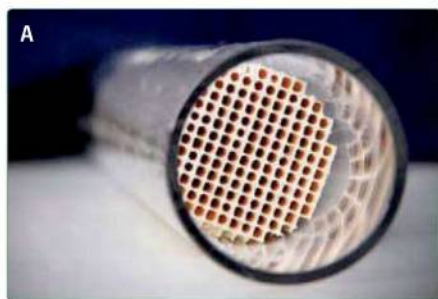
The catalyst developed by Kim *et al.* greatly reduces the amount of PGM in LNTs

while still maintaining their effectiveness for NO_x reduction from lean engines. This alternative technology will allow engineers greater flexibility as they work to develop better catalysts in a market where volatile PGM prices have made commercial introduction of fuel-efficient lean vehicles challenging. It is possible that these catalysts may allow lean-burn technology to be used with minimal added cost compared to conventional engines.

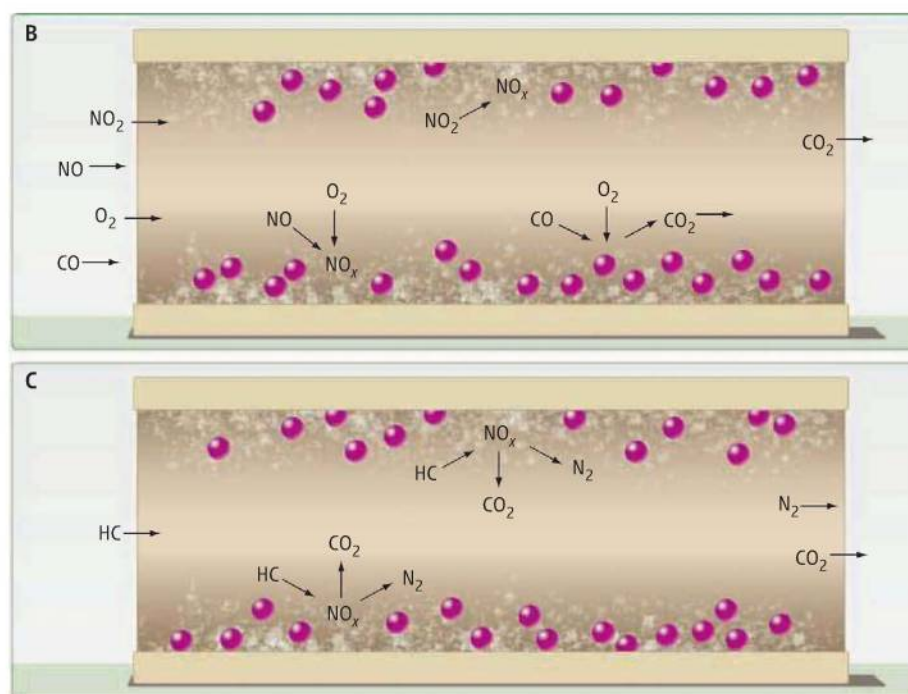
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10.1126/science.1187154



Lean but still clean. Engines that run lean on fuel can offer greater fuel economy but pose new challenges in the control of nitrogen oxide emissions that help create smog. (A) An image of a monolithic platinum-metal lean-trap catalyst. (B and C) A general schematic of the operation of lean NO_x trap catalysts when running during the (B) lean and (C) fuel-rich part of its cycle. When lean, oxygen-rich exhaust is produced, NO_x is absorbed on the catalyst surface. When fuel-rich exhaust is produced, NO_x is liberated and excess hydrocarbon helps reduce it to N_2 . The catalyst developed by Kim *et al.* may help decrease the levels of PGMs needed for these catalysts to be effective.



MEDICINE

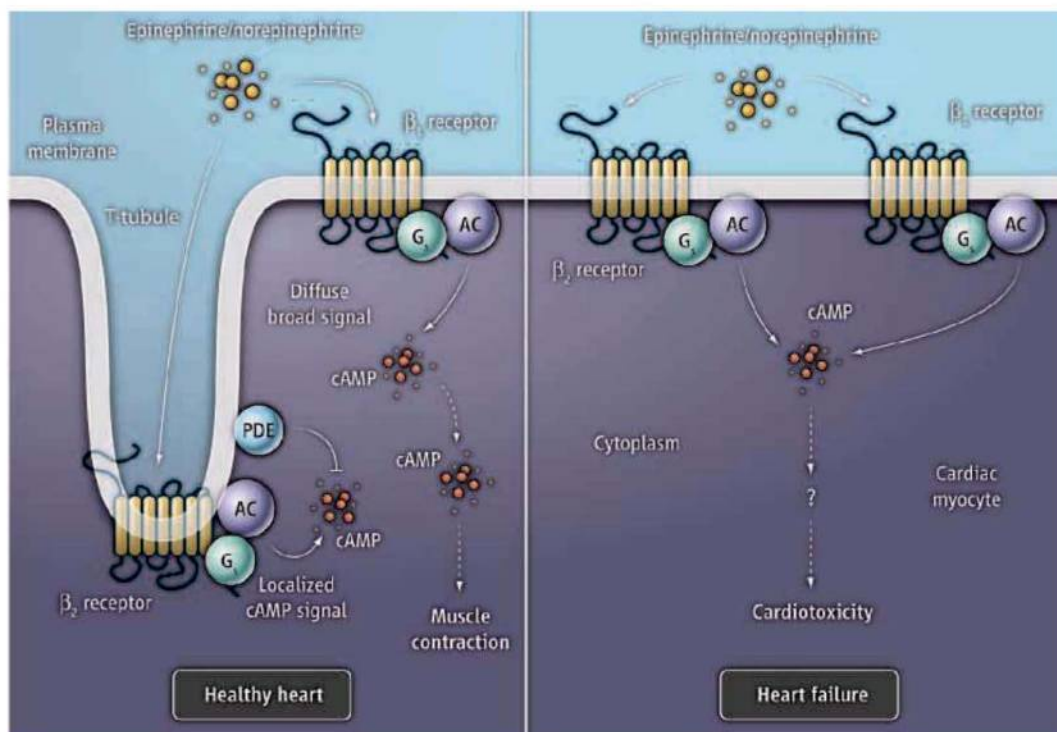
Refugee Receptors Switch Sides

Gerald W. Dorn II

Human heart muscle cells (cardiomyocytes) express two different types of β adrenergic receptor: β_1 AR and β_2 AR. Both respond to the same hormones and couple to the same biochemical signaling effectors, yet they elicit different cellular responses. On page 1653 of this issue, Nikolaev *et al.* (1) show that β_1 ARs are widely distributed at the plasma membrane (crest) of a normal cardiomyocyte, whereas β_2 ARs localize to transverse-tubular (T-tubule) regions at the cell surface, thus segregating their signaling. Remarkably, failing cardiomyocytes exhibit membrane remodeling that disrupts compartmentalization of β ARs. This may be a primary mechanism for the pathological effects of β AR signaling in heart failure and disease.

β_2 AR is the prototype of receptors that span the membrane seven times and are associated with a heterotrimeric guanine nucleotide-binding protein (G protein). Before 1990, the conventional view was that norepinephrine and epinephrine (adrenaline) secreted by neuronal and adrenal tissues activate β ARs that are coupled to the stimulatory subtype of G protein (G_s). This then triggers the enzyme adenylate cyclase to produce cyclic adenosine monophosphate (cAMP), a signaling molecule that was thought to diffuse throughout the cytoplasm to activate intracellular targets (2). However, this view changed after the description of macromolecular “signalosomes,” complexes consisting of β_2 ARs or other receptors and proteins that transduce and modulate the cAMP signal (3, 4). Such compartmentalization of components has emerged as a ubiquitous biological theme for orchestrating cell signal transduction to confer precision and specificity of response.

Upon stimulation, β_1 ARs increase the force and frequency of cardiac muscle contraction by



Receptor redistribution. Cell membrane remodeling in heart failure produces T-tubule loss, displacing β_2 ARs to cell crests where they exhibit β_1 AR-like nonlocalized cAMP signaling. A key question is whether β_2 ARs that translocate to cell crests exert the same cardiotoxic effects as β_1 ARs. AC, adenylate cyclase; PDE, phosphodiesterase.

inducing cAMP-dependent phosphorylation of factors that regulate the intracellular concentration of calcium. This consequently affects calcium-dependent proteins that control the contraction of myofilaments (see the figure). However, in heart failure, β_1 AR induces cell remodeling and programmed cardiomyocyte death, which contribute to the tissue deterioration seen in heart failure. Thus, treatment with β_1 AR antagonists protects against morbidity and mortality in heart failure, even though cardiac pump function is depressed (5). The pathophysiological role of cardiac β_2 ARs is less clear. Although they also couple to G_s and adenylate cyclase, cardiac β_2 AR-generated cAMP signaling is not transmitted to calcium-regulatory and myofilament proteins that control contraction. Instead, β_2 AR activity opposes β_1 AR signaling by switching sequentially from G_s to an inhibitory G protein (G_i) that blocks adenylate cyclase (6). This switching results in the production of different cAMP pools in the cardiomyocyte (7, 8).

Selective generation of intracellular pools of cAMP implies subcellular compartmentalization of signals. Indeed, fluo-

Disrupted localization of β_2 adrenergic receptors in cardiac cells may contribute to heart failure.

rescence resonance energy transfer (FRET) techniques for real-time live-cell imaging of intracellular signals have localized β AR-stimulated cAMP to discrete subcellular microdomains of the cardiomyocyte T-tubule system (4). T-tubules are plasma membrane invaginations containing many proteins that couple plasma membrane depolarization (excitation) to calcium-mediated myofilament shortening (contraction). β_2 ARs in normal cardiac myocytes are associated with T-tubules, thus generating spatially restricted cAMP production. This signaling is locally inactivated by phosphodiesterases located in T-tubules, which limits the diffusion of cAMP. By contrast, β_1 ARs produce cytosolic cAMP that diffuses the distance of a dozen or more myofilament sarcomeres to enhance contractility (8). Nikolaev *et al.* combined FRET with live-cell scanning ion conductance microscopy to colocalize β_2 ARs and their cAMP signaling events. By comparing spatiotemporal patterns of signaling in cardiomyocytes from normal and failing hearts of rats and from mice lacking either β_1 AR or β_2 AR,

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they observed redistribution of β_2 ARs from T-tubules to cell crests, the normal province of β_1 ARs. Consequently, β_2 ARs in failing cardiac myocytes acquired a pattern of broad cAMP signaling more characteristic of β_1 ARs. Because β_1 AR signaling is linked to cardiotoxicity in heart failure, the transition of β_2 ARs from a discrete to a generalized pattern of cAMP signaling may accelerate heart failure.

Altered excess β AR trafficking is characteristic of heart failure (9). Prolonged adrenergic stimulation induces receptor down-regulation by internalization and degradation. Even if internalized receptors are not degraded, internal sequestration uncouples them from signaling effectors (desensitization). But β AR down-regulation in human heart failure is specific to β_1 ARs, whereas β_2 ARs are spared. The net result is a change in the relative proportion of β_1 ARs versus β_2 ARs from ~4:1 in normal hearts to ~3:2 in heart failure (10). Furthermore, β_1 ARs that remain in failing hearts are largely uncoupled from G_s -adenylate cyclase-cAMP signaling because of desensitization. Receptor internalization also appears to be necessary for switching β_2 AR from G_s to G_i signaling (6, 11). Because the net effect of β_1 AR down-regulation and desensitization combined with β_2 AR switching to G_i signaling is to increase cardiac β_2 AR signaling at the

expense of β_1 AR signaling, these events have widely been assumed to benefit the failing heart (9).

A pathological role for T-tubule loss in heart failure has been attributed to compromised excitation-contraction coupling (12) and to the redistribution of voltage-dependent calcium channels (13). By linking β_2 AR redistribution in heart failure with plasma membrane remodeling and the loss of T-tubules, Nikolaev *et al.* add another layer of complexity to β_2 AR regulation in heart failure. Future studies will determine whether diffusible cAMP signals generated by displaced β_2 ARs couple to contraction and cell death pathways in the same manner as those generated by β_1 ARs.

Compartmentalization of β_2 ARs and restriction of their cAMP signals has not been observed in cells that normally do not have T-tubules or analogous intracellular membrane structures. T-tubules may therefore not only define β_2 AR signaling in normal versus failing cardiomyocytes, but also in cardiomyocytes versus other cell types. This establishes an imperative to study receptor signaling in cells directly relevant to the disease of interest and emphasizes necessary caution in extrapolating findings from one cell type to another. Likewise, there are important species differences between humans and rodents with respect

to T-tubule density, β_1 AR/ β_2 AR expression ratio, and in the capacity for β_2 ARs to undergo G_s -to- G_i signal switching. For these reasons, it is premature to consider changing the clinical use of compounds that block β ARs in human heart failure. However, the finding of Nikolaev *et al.* that β_2 ARs in heart failure can adopt pathological characteristics of β_1 ARs when displaced from T-tubules to cell crests does support the need to revisit prior clinical studies that concluded that β_1 AR-specific blockade is desirable in human heart failure.

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MATERIALS SCIENCE

Controlling Radiation Damage

Graeme Ackland

The ability of a material to resist radiation damage is determined by how well the microstructure can remove vacancies and interstitial defects in equal numbers. However, the exact processes by which this happens are poorly understood, and the search for promising materials has been largely heuristic. On page 1631 of this issue, Bai *et al.* (1) describe a mechanism in copper that suggests that nanomaterials in general might have exceptional radiation resistance. The authors' interpretation is supported by some previous experimental findings and suggests new directions for trial materials.

In recent weeks, the U.S. National Ignition Facility (the world's largest and highest-

energy laser) reached a threshold for fusion ignition (2). In France, the ITER fusion reactor is under construction (3). The possibility of building fusion power stations, producing energy in the form of vast numbers of energetic neutrons and alpha particles, is firmly back on the agenda. But a key problem remains: What will they be built of?

This problem is far from simple, because neutrons damage any structural material. This is an issue even for conventional fission power stations, but is particularly problematic for fusion, where the neutron fluxes are much greater and sensitive equipment (such as magnets or lasers) is required. Moreover, extracting energy from alpha particles is equivalent to helium implantation, which causes further material problems. Radiation damage is measured in "displacements per atom" (dpa)—that is, how often each atom is blasted away

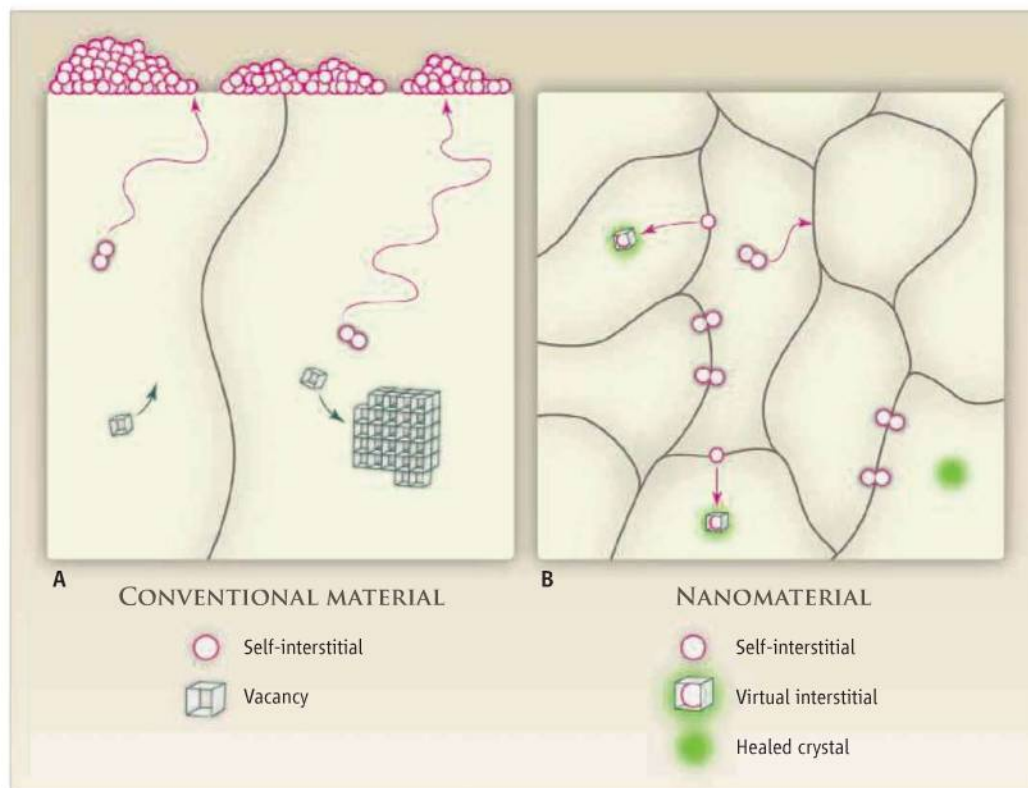
A self-healing mechanism may render nanomaterials highly useful for nuclear applications.

from its crystal site. A fusion reactor will produce many hundreds of dpa over its lifetime: Every atom in its structure will be removed hundreds of times. Materials must be found that can self-heal this extent of damage.

Primary damage takes the form of interstitial atoms and vacancies. Healing occurs when these recombine and annihilate. In most materials, however, the interstitials move quickly to the surfaces, causing the material to swell while leaving the vacancies behind. Furthermore, these point defects can aggregate (see the figure, panel A), forming larger obstacles to dislocation motion and causing hardening and embrittlement.

Nuclear materials must also be designed with an eye to decommissioning. The materials should therefore not contain atoms that will become radioactive under irradiation. This and other features of little interest in

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Heal thyself. In a conventional material (A), interstitial defects caused by irradiation quickly move to the surface, causing swelling. Vacancies slowly agglomerate, forming immobile voids that embrittle the material. Bai *et al.* show that in a nanostructured material (B), the interstitial defects get stuck in boundaries. Virtual interstitials re-emitted from boundaries annihilate with vacancies, leaving a healed crystal.

most engineering applications—such as the probability that a particular atom will absorb a neutron, becoming highly energetic and possibly radioactive—become important. The constraints rule out whole classes of conventional structural materials.

Furthermore, radiation damage is not an equilibrium phenomenon. This complicates the execution of experiments and the interpretation of the results because the time and dose dependence of radiation damage are not straightforward. As a complement to experiments, researchers are increasingly turning to computer simulation to uncover the atomic-level processes involved and understand how materials will behave over the lifetime of a reactor or storage facility.

Nanomaterials have been shown to have excellent radiation resistance in some cases, exhibiting very low swelling and retaining ductility despite being irradiated (4, 5). Bai *et al.* now report a theoretical explanation for this discrepancy. By performing a temperature-accelerated molecular dynamics simulation (6), they are able to simulate their material for times on the order of a microsecond. They show that the grain boundaries that are prevalent in nanomaterials can capture interstitials and then fire them back into the lattice to destroy

any vacancy that comes within a few nanometers of the grain boundary (see the figure, panel B). This efficient recombination method works even at low temperatures, where the vacancies cannot move. Because nanomaterials have a high proportion of boundary to bulk, a majority of the vacancies will be located within this annihilation range of a boundary.

Traditionally, it had been assumed that materials with very low vacancy mobility are unable to self-heal and would thus be especially susceptible to damage. The existence of a nanomaterial self-healing mechanism means that such materials may be ripe for further investigation.

Grain boundaries and interfaces have long been known to act as sinks for defects of all types. Generally this has been seen as a detriment to material performance, due to grain boundary embrittlement by elements such as helium, sulfur, and phosphorus. Control of nanostructures opens the possibility of creating materials with inclusions that act as alternative sinks for embrittlors. Carbon nanotubes, with their general strengthening properties and internal surfaces, are particularly attractive for use as “nano-dustbins.”

A practical difficulty with nanomaterials is that they tend to recrystallize under irradiation,

losing their nanometer-scale structural features. However, Bai *et al.*'s mechanism is very general and may also apply at interfaces between different materials. Nanoparticles of yttrium oxide, dispersed in ferritic steel to improve creep resistance, have also been shown to improve radiation resistance (4), and a similar mechanism could apply there. A large European Union-funded project is investigating this material (7). Misra *et al.* have shown that nanolayered niobium-copper composites show a similar resistance to damage, and because these metals are immiscible, the nanostructure is largely unaffected (5).

This study also touches on an older controversy: whether grain boundaries have a unique structure (8). Bai *et al.*'s hypothesis is that the interstitial defects can be absorbed (loaded) into the boundary, then re-emitted to annihilate a vacancy. The well-defined defect created by “loading” would not be definable if the boundary had no well-defined structure, because the defect would then be indistinguishable from the rest of the boundary. However, even

in a disordered boundary, a similar mechanism could work because it could emit interstitials without increasing its energy.

The calculations reported by Bai *et al.* are for a small sample of boundaries in a single model material, copper, but the mechanism that they discovered is highly promising. Much remains to be done to identify a commercially viable nanomaterial for use in nuclear reactors, but the potential commercial benefits of extending safe lifetimes are huge, making nanomaterials a highly promising target for future studies in this area.

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SPORE* SERIES WINNER

Addressing Science Teacher Needs

Nancy P. Moreno† and Deanne B. Erdmann

A pair of Web sites enhances teachers' content knowledge and teaching repertoire through asynchronous approaches.

Given the pace at which the scientific landscape changes, even practicing scientists can find it difficult to keep up with advances outside their fields of specialization. Imagine the daily challenge faced by classroom science teachers, who are trying to remain current with a broad range of scientific content and to incorporate the new information into existing curricula. This integration requires a depth and breadth of science knowledge not provided by the professional development available to most elementary, and even some secondary, school teachers.

In the United States today, there are 250,000 K–12 (kindergarten to high school) math and science teachers and 1.5 million elementary school teachers (many of whom provide science instruction) (1, 2). Their needs vary widely, and most have insufficient access to quality continuing education and teaching resources. Secondary-school science teachers need up-to-date content and training in laboratory techniques, such as how to use a micro-pipettor. Elementary teachers need help with basic understandings and teaching approaches across a wide range of topics, from earthquakes to the carbon cycle. These challenges are heightened in schools with significant populations of economically disadvantaged and at-risk students, where teachers tend to be less prepared to teach science (3).

BioEd Online (www.bioedonline.org) and K8 Science (www.k8science.org) are Web sites developed by Baylor College of Medicine (BCM) to address teachers' needs for accurate, timely science information and teaching materials. The sites capitalize on the Internet's capacity to reach many users at a relatively low cost. As 97% of U.S. schools report having broadband Internet connections, lack of access is no longer a major concern for potential teacher users (4).

BioEd Online was established in 2004, as a resource for biologists making a career transition, via alternative certification, to secondary school science teaching. The site proved useful and was expanded to serve a wider audience, including all life-science teachers, undergrad-

	Multiformat Teaching/Learning Tools						
	Curriculum modules	Semester courses	Content workshops	Demonstration workshops	Podcasts plus lessons	Teacher guides	Lecture series
Individual resources							
Video presentation/lectures	•	•	•	•			•
Annotated, downloadable PowerPoint slides	•	•	•	•		•	•
Podcasts					•		
Biology news		•	•				
Hot-topics essays with slide sets			•				
Interactive problems and self-assessments		•	•				
Video lesson demonstrations	•		•	•			
Video lab techniques demonstrations	•	•		•			
Science lessons by grade level and topic	•			•	•	•	
Resources for use with students (photos, videos, text)	•				•	•	

uate faculty, home-schooling families, and the general public. BioEd Online enables an instructor to learn about a topic such as light microscopy, to download a related lesson, and watch a demonstration on how to teach the lesson, all within a single Web site.

BioEd Online's asynchronous (any time, anywhere) approach allows large numbers of users to select and access specific online information and resources that meet their own immediate needs (see table, above). For example, a chemistry teacher recently reassigned to teach introductory biology might want to complete a short course on classical genetics. Another teacher might seek fresh ideas on the concept of "calorie" for tomorrow's lesson. We have made a deliberate effort to provide learner-specific information at the moment it is needed.

Recognizing the need to support science instruction in earlier grades, BCM launched a sister site, called K8 Science, in 2007. Both sites now offer a wide variety of materials that help teachers deepen their expertise and teach in ways that promote students' use of scientific evidence, engagement in scientific discourse, development of science knowledge, and excitement about science. For example, a K8 science lesson comparing the behavior of water drops and oil drops helps students understand water chemistry. Before conduct-

ing that lesson, teachers can review related concepts with a short course on the site.

Content on both sites is guided by an editorial board with diverse scientific and educational expertise. New resources are reviewed by at least two editors for content and educational appropriateness. Specialized reviewers are recruited as needed. Site content includes more than 80 streaming video presentations of current science information, instructional strategies, and lesson demonstrations; 785 downloadable PowerPoint slides with notes; biology news; podcasts; online courses; and discussion forums. Content is organized by resource type, and, where appropriate, by topic and grade. Some materials are indexed for multiple grades, and also may be incorporated into courses or units.

BioEd Online and K8 Science were accessed by more than 1.5 million users during the past year, and the numbers continue to grow. During a typical 30-day period (20 September to 21 October 2009) the sites received 162,400 active visitors (5400 per day), who viewed 594,000 pages and downloaded 16,620 lessons and related files (5). Because teachers access the sites through different Internet service providers, it is not possible to document how many users are in schools. However, the number of lesson downloads suggests that teachers are using

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*SPORE, Science Prize for Online Resources in Education; www.sciencemag.org/special/spore/. †Author for correspondence. E-mail: nmoreno@bcm.edu.

the sites. About 63% of users are identifiable as from the United States.

BioEd Online and K8 Science offer a growing number (over 120) of free downloadable (PDF) lessons and science inquiry modules developed by BCM educators and scientists for use with students. Most of these teaching materials were produced by federally funded curriculum development projects and field-tested in schools with teachers and groups of 150 to more than 1000 students. In recent field tests, students instructed by teachers using BCM-developed lessons showed statistically significant increases in science content knowledge, as compared with students in similar classroom settings who were not instructed by teachers using the materials (6, 7).

Web-based dissemination reaches large audiences at lower cost and enables rapid distribution of programs and products. This efficiency contributed to the success of the Butterflies in Space project, a collaboration of BioServe Space Technologies (University of Colorado), BCM, the U.S. National Space Biomedical Research Institute, and the U.S. National Aeronautics and Space Administration (NASA) (see the figure, below). Lesson materials, photos, and videos of Painted Lady butterflies living on the International Space Station during November and December 2009 were posted on BioEd Online and K8 Science. Students were able to access this content in near real time and then design their own experiments to compare space-based organisms with specimens living in their own classrooms. Close to 2900 teachers (representing 172,000 students in 23 countries) registered their classes to participate in the project, which offered a first-hand view of the butterflies' life cycle and attempts to fly in microgravity.



The Butterflies in Space project. A recently emerged Painted Lady butterfly spreads its wings on the International Space Station, amid specks of floating waste. BioEd Online disseminated related teaching materials and photos to classrooms around the world.

About the Authors



Nancy P. Moreno, editorial director of BioEd Online and K8 Science, received a Ph.D. in biology from Rice University. She is Professor of Allied Health Sciences and Family and Community Medicine and is senior associate director of the Center for Educational Outreach at BCM, where her work focuses on science education partnerships, including the use of Web-based approaches.

Deanne B. Erdmann received her B.S. and M.S. in biology from Stephen F. Austin State University. She was a biology teacher for 29 years and science department chair for 9 years before joining the Center for Educational Outreach at BCM, where she is assistant professor of Allied Health Sciences and managing editor of BioEd Online and K8 Science.



Partnerships expand the breadth and depth of resources on BioEd Online and K8 Science. Members of several BCM departments have provided presentations, content for courses, or editorial review. Partnerships with *Nature* and EarthSky Communications have allowed us to make content written for general or scientific audiences more useful to teachers by linking it to education standards and lessons. We are collaborating with the Houston Independent School District to enhance a year-round, face-to-face elementary teacher professional development program with customized online science content and lessons. The Web-based resources are matched, by grade level and topic (such as "magnets" or "animal behavior"), to the district's entire elementary school curriculum.

The most comprehensive offering thus far on BioEd Online is Genes, Health, and Society, a complete asynchronous course designed for high school teachers and undergraduate students. It covers a range of genetics topics, including biomedical applications of genomics. The course's first module was piloted with 28 undergraduate "pre-med" majors, 27 of whom reported that they were either "satisfied" or "very satisfied" with the overall experience. Learning gains demonstrated by students on matched before-and-after multiple-choice assessments were statistically significant. We are using the Genes, Health, and Society model to develop courses for teachers on cell and molecular biology and physical science, earth and space sciences, and environmental health science.

The challenges facing the U.S. science education community are

of national importance. How do we bolster the science knowledge and teaching skills of thousands of teachers, each with unique professional development and classroom teaching needs? How do we accommodate multifarious local requirements for curriculum, content, and resources? And how do we address these issues in cost- and time-efficient ways? Online approaches are not the only answer to our nation's science education challenges, but they represent an important strategy for our collective response as a science community.

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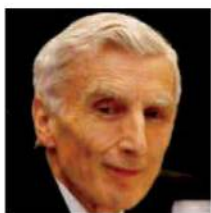


AAAS ANNUAL MEETING

Science Leaders Urge New Effort To Strengthen Bonds with Public

SAN DIEGO—Public engagement is perennially a top issue for the global science and engineering community, but with political attacks against climate science escalating and polls reflecting a decline in public confidence, the issue has grown more critical. At the recent AAAS Annual Meeting, science leaders concurred that more effort and creativity are needed to meet the goal.

But at a press briefing on climate change, organized by the U.S. National Academy of Sciences and AAAS, an eminent panel of science leaders made clear the complexity of the challenge. If science is based on reason and evidence, they asked, how should it respond when economic stress and a concerted campaign of distortion undermine the public's belief in climate change and trust in science?



Lord Martin Rees

Climate change faces "special problems," said Lord Martin Rees, the eminent cosmologist and president of the Royal Society. "It's diffuse and international—you can't do anything about it unless all nations move together. It's also remote in time: The consequences will only impact seriously on the next generation, not us, which makes it very hard to get the public exercised on the need to do something."

Ralph Cicerone, president of the National Academies of Science and chair of the National Research Council, warned that rising public skepticism on climate change apparently has "spilled over into other kinds of science."

The issue of public engagement was the central theme of the Annual Meeting, which convened here from 18 to 22 February under the banner "Bridging Science and Society." In his invitation, AAAS President Peter Agre underscored the theme.

Building stronger bonds with the pub-



Building a bridge. The *San Diego Union-Tribune* published a series of three commentaries from AAAS during the Annual Meeting in February.

lic requires "every scientist and engineer to make their work both beneficial and understandable, and...society to discover again the excitement and hope that research and its findings offer," said Agre, who shared the 2003 Nobel Prize in chemistry. "It is a call to action that resonates around the world."

A workshop in science communication was offered to scientists and engineers at the meeting. Some three dozen special briefings were staged for corps of U.S. and international science journalists. Thousands of people attended Family Science Days, plenary addresses, and other free public events.

But researchers recently have noted signs of a strained relationship with the public on several important issues—climate and energy, embryonic stem cell research, and evolution.

Those and other issues were addressed in three commentaries written by Agre and prominent coauthors and published by the *San Diego Union-Tribune* during the meeting.

"In an era of incredible opportunities and profound problems, our nation can only thrive if decisions are shaped by a science-literate public and well-informed policymakers," Agre wrote with Alan I. Leshner, the AAAS CEO and executive publisher of *Science*. "Those may be the defining challenges for our research enterprise in the 21st century."

Some at the meeting focused on a more immediate challenge: responding to the leak or theft of hundreds of e-mails from the University of East Anglia's Climate Research Unit and the discovery of a handful of errors in the

massive 2007 report of the Intergovernmental Panel on Climate Change (IPCC), including one that misstated the speed at which Himalayan glaciers are melting.

Critics have cited the e-mails and the errors in broad attacks against the validity of climate change research. Recent polls show that, in a matter of months, public belief in human-caused climate change has dropped sharply.

Former AAAS President Jane Lubchenco, now head of the U.S. National Oceanic and Atmospheric Administration, told reporters the errors were an "embarrassment" and a "wake-up call" for the IPCC.



Jane Lubchenco

Then-AAAS Chairman James J. McCarthy, who served as a co-chair of an IPCC working group that helped produce the panel's 2001 report, noted that some unfavorable public opinion is likely linked to the nation's economic distress. But the IPCC errors should have been caught, he said.

"Our institutions are not as nimble as they should be in acknowledging...that we really do need to endeavor to make information more readily available and when errors occur, to correct them immediately and explain their origin," McCarthy added at the AAAS briefing.

Cicerone agreed. "We have to address our fundamentals in any case as we continue to improve science," he said. "So let's do it, let's introduce these improvements and...hope it will set a new level of transparency and potential trust."

INTERNATIONAL

S&T Policy Fellows Aid Haitian Recovery

A few weeks after Haiti's devastating January earthquake, Allegra da Silva was on the streets of Port-au-Prince, interviewing people about latrines. Jay Graham was helping assess water and sanitation needs in the camps of the newly homeless. Adam Reinhart was planning for the day when Haitian farmers could use their trucks to bring mangoes to market instead of ferrying food aid to the battered capital.

The current and former AAAS Science & Technology Policy Fellows, all working for the U.S. Agency for International Develop-

ment, are seasoned scientists and engineers whose technical skills and policy experience made them valuable both in Haiti's initial relief efforts and as the country shifts toward long-term recovery.

Scientists can "bring rigor and data" to understanding Haiti's needs, said Graham, in his second year as an S&T Fellow. "This is disaster response, but it's also an issue of strategically moving forward, and connecting what's done in disaster relief with something larger and longer in scope."

Launched in 1973, the AAAS S&T Policy Fellowships have sent more than 2000 scientists and engineers to work in Congress and nearly 20 executive branch agencies and departments. The efforts of those working in Haiti "are indicative of the skills that Fellows can offer by applying their knowledge in real-world situations to address challenges and bring about positive change," said Cynthia Robinson, director of the Fellowships.

Da Silva, an S&T Fellow with a Ph.D. in environmental engineering, came to Haiti as part of a USAID Rapid Environmental Assessment team. The team interviewed Haitians about urgent needs and ongoing challenges in coordinating relief efforts to provide clean water, garbage collection, and shelters.

She talked with people about everything from "flying latrines"—plastic bags used in lieu of toilets—to their congested tent cities made of wooden poles and bedsheets. "Lives come first," said da Silva, "but we want to support ways in which the relief effort can mobilize resources with the least environmental impact and least negative impact on livelihoods."

For Reinhart, Haiti is familiar territory; he visited twice before the earthquake to talk with farmers about roads, fertilizer, and soil erosion. The former S&T Fellow, now working on economic security issues with USAID's Haiti Task Team, helped establish USAID's WINNER program, which will invest \$126 million over 5 years to strengthen Haitian agriculture.

In addition to the damage around Port-au-Prince, said Reinhart, "the earthquake has exacerbated existing infrastructure problems countrywide." Refugees fleeing the capital have placed added strain on the infrastructure of rural areas and smaller cities.

Graham had one week to prepare after getting the call from his boss John Borrazzo—another former S&T Fellow—to join a USAID team planning water and sanitation solutions for burgeoning camps of displaced people

in Port-au-Prince. Arriving a month after the quake, Graham found busy streets and markets—but the environmental health advisor also saw long-term water and sanitation needs that will require significant investments.

Scientists have much to offer in Haiti, but they have to conduct research in a way that doesn't burden the relief effort, said Olga Cabello, a former S&T Fellow who now directs the International Development Seismology initiative at IRIS, a consortium of U.S. universities that supports the global collection and open exchange of seismological data.

IRIS is working with federal agencies, the United Nations, and universities on "recommendations that can be used to reconstruct the country and the community at every level," said



Urgent needs. AAAS S&T Policy Fellows interviewed Haitians about clean water and sanitation after the January quake.

Cabello. "You do not want to reconstruct vulnerability—you're rebuilding for resilience."

Some of the researchers will return soon to Haiti to continue the job of recovery. "Everybody is working really long hours there—it was pretty intense," said Graham, who put in 15-hour days during his first visit. "This work, I do believe in it. You can create the right incentives so people can organize themselves to solve problems."

—Molly McElroy, Benjamin Somers, Edward W. Lempinen, and Becky Ham

SCIENCE AND HEALTH

Personalized Medicine: How Physicians Fare

Doctors are already using genetic data to customize patients' treatments, but they could soon be overwhelmed by logistical and legal issues arising from inexpensive genome screening, experts said at a colloquium cosponsored by AAAS.

Within the next two years, people will be able to get their entire genomes sequenced for less than \$1000, the speakers said. But many doctors lack the tools to use this information, they cautioned, and could face malpractice suits if they ignore or misinterpret the data.

The \$1000 screen "is a 10-mile-wide asteroid heading toward us," said Hank Greely, a Stanford University law professor. "We don't have a clue how we're going to handle it."

The conference, held 8 to 9 March and cosponsored by the Food and Drug Law Institute, the Sandra Day O'Connor College of Law at Arizona State University, and the Mayo Clinic, was the third personalized medicine colloquium organized by AAAS in 2009–2010. The latest event offered a unique perspective on changes that may be in store.

Personalized medicine is "a very slow train," said Lee Hartwell (who received the 2001 Nobel Prize in Medicine), in part because it relies heavily on a small number of genetic markers that predict disease.

Hartwell, codirector of the Center for Sustainable Health at Arizona State, said the risk of disease associated with these markers often isn't large enough to help doctors. "I don't have a lot of hope of taking your DNA sequence and predicting your disease susceptibility," he said. "Family history is still a better predictor than that for most common diseases."

Protein biomarkers are much more useful than genes for the doctor in intensive care who needs to know whether a drug is causing a patient's kidneys to shut down, said Prasad Devarajan, the director of nephrology and hypertension at Cincinnati Children's Hospital. It's a problem that's "crying out for personalized medicine," he said, since drug toxicity varies considerably from patient to patient.

For example, Devarajan has studied the protein NGAL, which is produced by injured kidneys. It can be detected in urine days before other signs of poisoning appear, he said, and that makes it a good example of how biomarkers can be used to tailor real-life treatments.

For Donald McAlpine, the trove of genetic data related to antidepressant medication is a "scouting report" that "makes prescribing more complex, but ultimately better." McAlpine, an assistant professor of psychiatry at the Mayo Clinic, underscored the point by unfurling a scroll of genetic variations so long that it spilled over the edge of the podium. "All this information is hard for the busy clinician to manage," he noted wryly.

But he and others suggested that the matter has serious ramifications. "Health care professionals are likely the most vulnerable to liability risks associated with personalized medicine," said Gary Marchant, a professor of law and life sciences at Arizona State.

Many physicians unfamiliar with genetics, he noted, may soon encounter patients who want their doctors to use genetic data in treatment decisions.

Kenneth Offit, chief of clinical genetic service at Memorial Sloan-Kettering Cancer Center, led a 2003 study that found some women with mutations in the *BRCA*

gene might reduce their risk of several cancers if their ovaries were removed. Eighteen months later, he said, “we saw the first malpractice suit” against a doctor who had not removed the ovaries of a patient with the mutations.

“This meeting revealed the still enormous gap between our ability to generate large

volumes of data and to offer targeted treatments,” said Mark S. Frankel, director of the AAAS Scientific Freedom, Responsibility and Law program. “The gap is the greatest challenge we face in achieving a level of personalized medicine that makes a real difference in health care.”

—Becky Ham

Results of the 2009 Election of AAAS Officers

Following are the results of the 2009 election. Terms began on 22 February 2010.

General Offices

President-Elect: Nina V. Fedoroff

Board of Directors: Stephen Mayo, Sue V. Rosser
Committee on Nominations: Pamela Bjorkman, Tom Lovejoy, Barbara Schaal, Maxine Singer

Section on Agriculture, Food, and Renewable Resources

Chair-Elect: Charles Arntzen

Member-at-Large: Jan Leach

Electorate Nominating Committee: Shauna Somerville, Jean Steiner

Section on Anthropology

Chair-Elect: Cynthia Beall

Member-at-Large: Nina Jablonski

Electorate Nominating Committee: Stephen Leigh, Kathleen O'Connor

Council Delegate: Pat Shipman

Section on Astronomy

Chair-Elect: John Huchra

Member-at-Large: Margaret Meixner

Electorate Nominating Committee: Giuseppina “Pepi” Fabbiano, Jay Pasachoff

Council Delegate: Lori Allen

Section on Atmospheric and Hydrospheric Sciences

Chair-Elect: Otis Brown

Member-at-Large: Mary Anne Carroll

Electorate Nominating Committee: James H. Butler, John W. Farrington

Section on Biological Sciences

Chair-Elect: Mary Lou Guerinot

Member-at-Large: Loren Rieseberg

Electorate Nominating Committee: Charles Aquadro, Jennifer Stone

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Section on Chemistry

Chair-Elect: Cynthia J. Burrows

Member-at-Large: Ron Raines

Electorate Nominating Committee: Klavs Jensen, Timothy P. Lodge

Council Delegates: John Hartwig, Clark Landis, Jeanne Robinson

Section on Dentistry and Oral Health Sciences

Chair-Elect: Carolyn W. Gibson

Member-at-Large: Kenneth Yamada

Electorate Nominating Committee: Dennis Mangan, Mina Mina

Section on Education

Chair-Elect: Elizabeth Stage

Member-at-Large: Sean Decatur

Electorate Nominating Committee: Patricia Mabrouk, Marilyn Suiter

Section on Engineering

Chair-Elect: H. Vincent Poor

Member-at-Large: Gary May

Electorate Nominating Committee: Cynthia Bruckner-Lea, Richard Alkire

Section on General Interest in Science and Engineering

Chair-Elect: Judith E. Parker

Member-at-Large: Michael Isaacson

Electorate Nominating Committee: Deborah Illman, Bassam Shakhshiri

Section on Geology and Geography

Chair-Elect: Ellen Mosley-Thompson

Member-at-Large: Thomas Cronin

Electorate Nominating Committee: Bruce Molnia, Henry Pollack

Council Delegate: Thomas C. Johnson

Section on History and Philosophy of Science

Chair-Elect: Jane Maienschein

Member-at-Large: Sally Gregory Kohlstedt

Electorate Nominating Committee: David Hounshell, Audra Wolfe

Section on Industrial Science and Technology

Chair-Elect: Carol J. Burns

Member-at-Large: Kenneth A. Jackson

Section on Information, Computing, and Communication

Chair-Elect: Vinton G. Cerf

Member-at-Large: Maja J. Mataric

Electorate Nominating Committee: Maureen C. Kelly, Tom Pyke

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Chair-Elect: Mark Liberman

Member-at-Large: Jennifer Cole

Electorate Nominating Committee: Jean-Pierre Koenig, Joseph Salmons

Section on Mathematics

Chair-Elect: John Ewing

Member-at-Large: Mary Ellen Bock

Electorate Nominating Committee: Carl Cowen,

Robert Megginson

Council Delegate: Philippe Tondeur

Section on Medical Sciences

Chair-Elect: Edward Benz

Member-at-Large: Harriet Robinson

Electorate Nominating Committee: Lisa Guay-Woodford, Ruth Ruprecht

Section on Neuroscience

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Member-at-Large: Don Cleveland

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Member-at-Large: Ah-Ng “Tony” Kong

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Member-at-Large: Jolie Cizewski

Electorate Nominating Committee: S. James Allen, Katharine Gebbie

Council Delegates: David Gross, John Negele

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Member-at-Large: Jocelyne Bachevalier

Electorate Nominating Committee: Joe Martinez, Janet Werker

Section on Social, Economic, and Political Sciences

Chair-Elect: Kenneth Bollen

Member-at-Large: Julia Lane

Electorate Nominating Committee: David Collier, Nancy Lewis

Section on Societal Impacts of Science and Engineering

Chair-Elect: Rachel Levinson

Member-at-Large: Howard Gobstein

Electorate Nominating Committee: Angela Creager, Donna Nelson

Section on Statistics

Chair-Elect: Thomas A. Louis

Member-at-Large: M. Elizabeth Halloran

Electorate Nominating Committee: Marie Davidian, Nick Jewell



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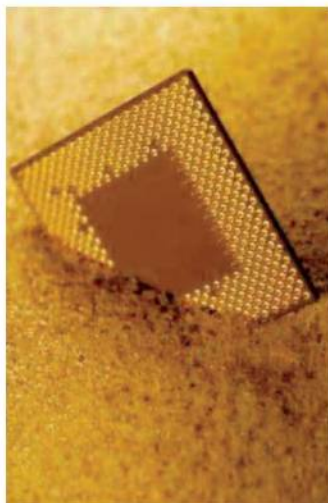
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INTRODUCTION

Looking Beyond Silicon

SILICON-BASED ELECTRONICS HAVE BEEN THE MAINSTAY OF THE INDUSTRY FOR several decades, a veritable powerhouse of the economy, driving technological breakthroughs that affect virtually all aspects of everyday life. Devices have gotten smaller, faster, more efficient, more powerful, and cheaper. However, the size of transistors—the building blocks of electronics—is approaching the limits of what can be done on a large-scale industrial basis. Has the time come when a replacement for silicon can no longer be avoided? If we look beyond raw processing power, might there be a future for silicon if it is given new capabilities?

On page 1600, Theis and Solomon set the agenda, describing the limits that silicon transistors have reached. They outline some of the different approaches being taken in developing a new transistor, still based on silicon but involving clever redesign and engineering of the basic structure. Summing up, they acknowledge that it may well be a materials issue—whether it is pushing the capabilities of silicon farther or developing new materials altogether.

Moving away from silicon, Takagi and Hwang (p. 1601) look at the richness of the electronic phases and enhanced functionality afforded by transition metal oxides. The properties of these strongly correlated electron systems can be dramatically affected by small changes in applied voltage or magnetic field, and can be used as memory storage or active devices.

Rogers and co-workers (p. 1603) review developments in making the electronics wearable or more transportable through a combination of flexibility, bendability, or stretchiness obtained by integrating inorganic and organic materials with suitable substrates. A recently published paper by Viventi *et al.* in *Science Translational Medicine* describes a method of using flexible electronics to measure the patterns of a beating heart.

Finally, Mannhart and Schlom (p. 1607) review the developments made with oxide interfaces. The junction of two insulating materials has been found to exhibit a vast array of different properties—metallic, superconducting, and magnetic—with the effects being tunable and devices scribable with an electric field. Mastering control over the interface properties is a formidable challenge, but doing so will offer unforeseen opportunities for technology and device function.

In a pair of News stories, Service (p. 1596) discusses an impending crisis in supplies of rare-earth elements and other materials vital to novel electronics and energy technologies and (p. 1598) describes advances in nitride semiconductors: materials that hold promise for fast-switching, heat-tolerant transistors, compact chemical sensors, and high-performance solar cells. *Science Careers* supplements the section with an online profile of a young British researcher working on nano-scale structures in gallium nitride.

Materials lie at the heart of all electronic devices. With the wide array of tricks used to coax silicon to do more and the wide range of new materials finding their way into electronic devices, the future looks to be more promising than the technology, gadgetry, and functionality we have seen thus far.

— IAN OSBORNE, MARC LAVINE, ROBERT COONTZ

Materials for Electronics

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Science

NEWS

Nations Move to Head Off Shortages of Rare Earths

Looming scarcities of a handful of essential elements could shake the electronics industry, unless manufacturers and mining companies develop more sources soon

PICTURE THE PERIODIC TABLE AS A CITY.

You've got your flashy downtown: gold, silver, and the like. You've got your industrial sector: iron and nickel. There are parks: carbon, oxygen, and nitrogen. And of course the "troubled" section: radionuclides. Then there are the forgotten, distant exurbs: the rare earth elements (REEs), lanthanum (element 57) through lutetium (element 71) along with scandium and yttrium. Sleepy no more, the exurbs have turned very desirable. In recent decades, REEs have become vital to a host of novel electronics and green-energy technologies. The trouble is that, while researchers are steadily inventing new applications for rare earths, the supply isn't keeping up—and users of REEs are feeling the pinch.

Today, China supplies more than 97% of all REEs, and increasingly the products from which they are made. Those products span a wide swath, ranging from phosphors in electronic displays, to magnets in disk drives, cell phones, and MRI machines, and motors in missile guidance systems. In 2000, about 60,000 metric tons of rare earth oxide ores were mined worldwide. By 2014, that num-



ber is expected to grow to 200,000 metric tons.

China's production of REEs has been growing steadily over the past decade. But because its domestic demand for the elements has been growing even faster, the country's REE exports have dropped from 75% of the total produced to 25% (see figure, p. 1597). For a handful of elements—neodymium, dysprosium, terbium, and yttrium—China is expected to use all it can produce sometime between 2012 and 2014, leaving the rest of the world out in the cold (*Science*, 11 September 2009, p. 1336).

"We are going to have to start acting quickly, or we will be in big trouble," says Ed Richardson, the sales and marketing manager for Thomas & Skinner in Indianapolis, one of the few makers of high-intensity magnets left in the United States, which relies on REEs to make its products. And with ever more applications on the horizon, Richardson says, "the problems we are seeing today are only going to get worse over time." REEs aren't the only concern. Supplies of other metals vital to electronics and green technologies—such as indium and lithium—are also tightening as industrial uses for these materials skyrocket.

Rare earths are not rare. They are ubiquitous in soils around the globe. But unlike gold and silver,

which are heavily concentrated in particular ore deposits, rare earths are sparse. The world's richest veins of REEs contain only 4% to 9% of the elements. Most deposits have 1% or less of the elements—too little to make their processing economical, says Mark Smith, chief executive officer of Molycorp Minerals in Greenwood Village, Colorado, the only remaining rare-earth mining company in the Western Hemisphere.

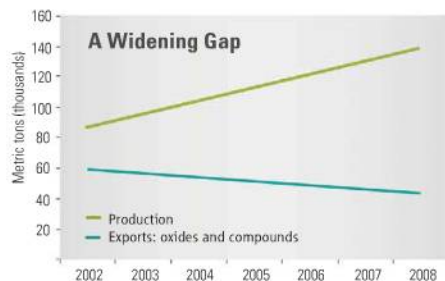
In some cases, mining companies are responding. For example, in the United States, Molycorp plans to reopen a mine in Mountain Pass, California, expected to produce up to 20,000 metric tons of rare earth oxides by 2012. In Canada, Great Western Minerals Group and Avalon Rare Metals hope to tap vast rare-earth deposits in Saskatchewan and the Northwest Territories. However, rare-earth mining consultant Jack Lifton notes that these companies have not been able to secure all the funds they'll need to start operations.



Rare talent. The unique electronic structure of rare earths makes them vital for electronic displays, hybrid cars, and many other products.



CREDITS (LEFT TO RIGHT): ISTOCK PHOTO; IZMOSTOCK/ALAMY



New mining efforts are just the first step. Numerous rare earth oxides are invariably mixed together in ores. They must be separated and purified, reduced to metallic form, and then alloyed, cast, and shaped. All the Western businesses that used to do these extra jobs are gone and will need to be restarted.

Setting up this full suite can cost hundreds of millions of dollars and take up to a decade to accomplish, Lifton says. That's enough to scare away most investors, who are interested in shorter term payoff. Investors also worry about a business that can be so easily manipulated by a single government, says Jeff Green, president of J.A. Green and Co., a government relations firm in Washington, D.C., specializing in rare earths. China seems unlikely to flood the market today, but it did just that in the 1980s and 1990s, driving most Western producers out of business. "It creates a really unstable investment situation," Green adds.

Green, Lifton, and others say plenty can be done to get over such hurdles. Last month, magnet industry leaders in the United States sent a letter to John Holdren, director of the U.S. Office of Science and Technology Policy in Washington, D.C., calling on the Obama Administration to take prompt action to restore rare-earth mining and processing in the United States and other Western countries. The recommendations included establishing short-term stockpiles of rare earths critical for defense needs and having the U.S. Department of Energy set up a \$2 billion loan-guarantee program to help Western mining companies build new mining and processing facilities. Congress has already drawn up a bill to push such efforts, though it has yet to be introduced.

Such efforts could get a boost early next month when the Government Accountability Office (GAO) is scheduled to release an interim report on the vulnerability of defense applications to rare-earth shortages. If GAO determines that particular rare earths are vital to national security, it "could really get the ball rolling" in prompting government

Losing ground. China produces nearly all the world's rare earths. But as uses for the elements increase, China's exports of them are declining.

agencies to back REE mining and stockpiles, says Gareth Hatch, director of technology at Dexter Magnetic Technologies in suburban Chicago and editor of the RealMetalBlog.

Some manufacturing companies aren't waiting for U.S. and Canadian mining companies to take action. Toyota recently made a deal with a rare-earth mine in Vietnam, securing supplies of neodymium and lanthanum. In a single

Prius, Toyota uses a kilogram of neodymium for its electric engine and 10 kilograms of lanthanum for its nickel metal hydride battery. In January, a Toyota supplier also formed a partnership with a mine in Argentina to supply lithium for batteries for its next generation of plug-in hybrid vehicles.

Lifton says other Western companies and governments need to act decisively, or they will find themselves frozen out of the market. "We have 3 to 5 years to get it done," he says. "And we haven't even started yet."

—ROBERT F. SERVICE

Mission: Irreplaceable?

SCIENTISTS ARE SCRAMBLING TO DEVELOP SUBSTITUTES FOR SCARCE ELEMENTS

critical to industry. But the myriad uses of the elements make the effort an uphill struggle. "It's worthwhile to try and find replacements," says Peter Dent, vice president for business development at Electron Energy in Landisville, Pennsylvania. "But it won't be easy."

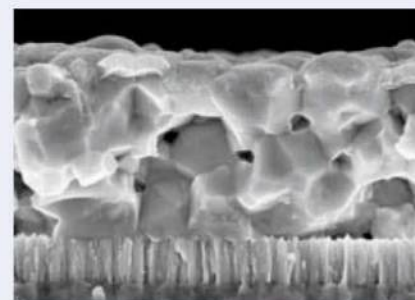
Dent's company makes high-powered magnets used in electrical generators. Magnets have increased in strength more than 100-fold over the past century—most dramatically in the 1970s and 1980s, when researchers added samarium and neodymium to magnet alloys. The rare earths fortify the alloys against outside magnetic fields, says Bill McCallum, a materials scientist at Iowa State University and Ames Laboratory in Ames, Iowa. Such fields can easily depolarize (demagnetize) a magnetic material such as iron, McCallum notes, because iron lacks strong "anisotropy," or preferred orientation of its north and south magnetic poles. Adding neodymium, which has a strong anisotropy thanks to its electron structure, creates a much more powerful alloy. That property will be hard to replace, says Jack Lifton, a rare earths consultant in suburban Chicago, Illinois.

Nevertheless, researchers are trying. George Hadjipanayis, a physicist at the University of Delaware, Newark, says he and colleagues recently received funding to develop high-strength magnetic materials made from neodymium, iron, and boron nanoparticles that they developed in 2007. Neodymium-iron-boron magnets are the strongest conventional magnetic materials; mixing the elements at the nano-scale should yield at least as much magnetization with less neodymium, Hadjipanayis says. First, though, the team must make large three-dimensional collections of such nanoparticles and align their crystallographic axes—so far, a tall order.

Other efforts are further along. Solar cell makers have been working for years to perfect semiconductor alloys made from ultrathin films. The most successful use indium, the price of which has spiked to more than \$1000 a kilogram in recent years. Researchers have made alternative versions from abundant elements, such as copper, zinc, tin, and sulfur (CZTS). And last month, researchers at IBM reported in *Advanced Materials* that they had increased the sunlight-to-electricity efficiency of CZTS cells by 40%, to 9.6%—less efficient than indium-containing cells but close to the threshold of 10% considered critical for commercialization.

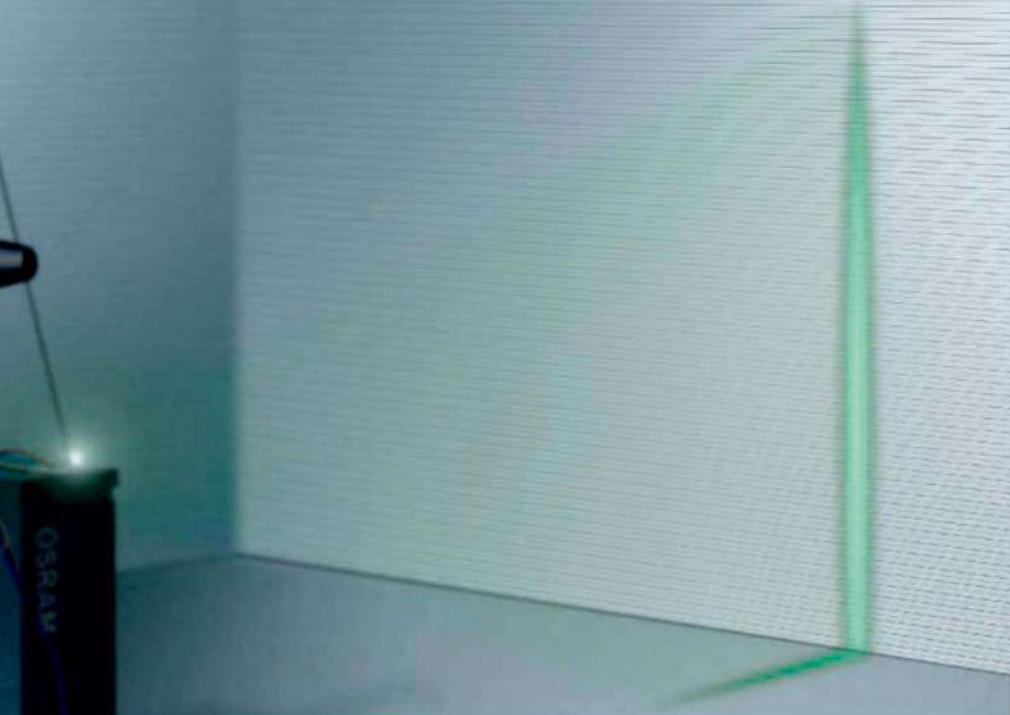
Researchers around the globe have also been toiling to replace indium used in transparent conductors in electronic displays. Last year, a team led by materials scientist Yang Yang of the University of California, Los Angeles, reported in *Nano Letters* that it had developed highly conductive transparent films by layering graphene (single-atom-thick sheets of carbon) and carbon nanotubes. The films aren't as good as ITO yet. But an analysis last year by Nano Markets, a market research firm in Glen Allen, Virginia, suggests that novel transparent conductors could soon find widespread use.

—R.F.S.



New recipe. Experimental CTZS solar cells at IBM are rare-earth-free.





First light. Nitride-based green lasers made their debut in 2009 and may usher in ultrasmall full-color projectors.

NEWS

Nitrides Race Beyond the Light

Long prized for their optical properties, nitrogen-based semiconductors may take electronic devices into realms where silicon cannot tread

SIXTEEN YEARS AGO, SHUJI NAKAMURA HELD up a few shining blue lights before a packed scientific meeting hall in San Francisco, California, and the audience oohed, aahed, and even produced a few groans. Nakamura—then a materials scientist with the Japanese chemical company Nichia and now with the University of California (UC), Santa Barbara—had created the first high-brightness, blue light-emitting diodes (LEDs), beating many of those in the room to the punch. That success paved the way for the blue lasers used in modern “Blu-ray” DVD players, as well as white LEDs that are now poised to usher incandescent light bulbs into the trash bin of technological history.

All of these gizmos were made possible by advances in making nitrogen-based semiconductors, known as nitrides. Nakamura’s lasers and LEDs were made from gallium nitride (GaN) and other related semiconductor alloys. And advances in optical devices made from GaN continue at a rapid pace. Researchers in Germany and Japan reported last year, for example, that they had made the first ever green laser diodes from indium gallium nitride, an advance that could make possible a new generation of tiny full-color projectors. Researchers are reporting similar success with nitride-based electronic devices, including transis-

tors that work at high speeds and at high temperatures, novel solar cells, and ultrasmall chemical sensors.

“It’s a very exciting time for nitride electronics, and the performance is increasing very fast,” says Tomas Palacios, an electrical engineer at the Massachusetts Institute of Technology in Cambridge. Such devices have the potential to outperform better-known silicon electronics for a wide range of applications.



One big reason for this versatility, Palacios and others say, is that—in contrast to most other semiconductors—the electronic behavior of nitrides can be tuned over a wide range. Semiconductors can act both as conductors—in which electrons move freely—and insulators, in which electrons are locked in. All semiconductors can be switched on to become conductors simply by adding extra energy. This kicks electrons from their locked state—known as the valence band—into their mobile state, or conduction band. The difference in energy between valence and conduction electrons is known as the material’s band gap.

Silicon, for example, has a band gap of 1.1 electron volts (eV). Add that much energy and silicon’s stationary electrons will hit the road. But if you’d rather have a silicon device with a higher band gap—which could be useful for making a more efficient solar cell whose band gap matches the energy of incoming photons, for example—you’re stuck. Combining multiple cells, each tailored to capture a different part of the solar spectrum, typically produces more efficient devices overall. That’s something the nitrides can accomplish. Indium nitride (InN) has a smallish band gap of 0.6 eV, GaN’s is 3.4 eV, and aluminum nitride’s is 6.2 eV. But three-part alloys, such as indium gallium nitride, can be adjusted to hit any band gap between InN and GaN by varying the amounts of indium and gallium. The same goes for the three-part alloy of aluminum gallium nitride. “This gives us a high flexibility to design new optoelectronic and electronic devices,” Palacios says.

Nitride semiconductors have other advantages as well. For starters, they’re electron autobahns, allowing electrons to travel about four times as fast as in silicon. They are also extremely hard and able to withstand temperatures hundreds of degrees higher than silicon can, making nitride-based transistors and other devices better suited for a wide variety of applications such as electronics in automobile engines and devices that tailor the electrical voltages coming from the grid to particular appliances.

Protein probes. Each of these nitride-based sensors is capped with an antibody to detect breast cancer.

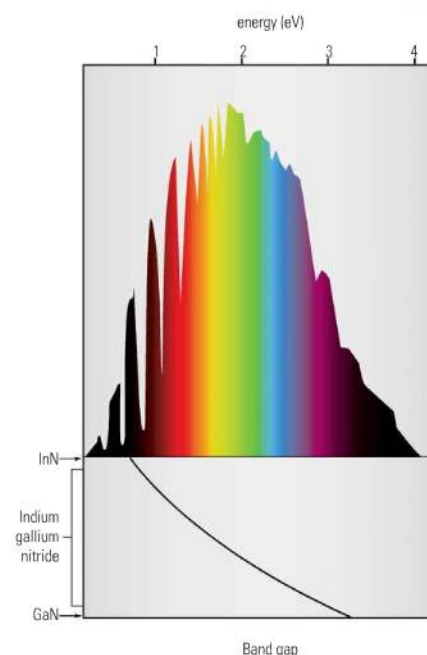
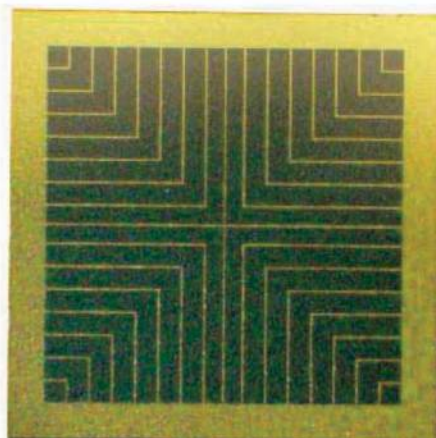
CREDITS (TOP TO BOTTOM): OSRAM PRESS PICTURE; H. T. WANG

On the minus side, nitrides can be hard to work with, partly because researchers haven't yet developed an ideal substrate on which to grow them. Silicon electronics are grown atop pizza-sized wafers of single-crystalline silicon. But because there has been no robust way to make large single crystals of GaN, most nitride electronics are grown on substrates such as sapphire and silicon carbide, which have differently spaced atomic lattices and other drawbacks. As a result, Nakamura and others struggled for years to make GaN LED and laser diodes, because the GaN wound up riddled with cracks and other defects that interfered with the performance of the devices.

But recent advances suggest that help could be on the way. For example, over the past 2 years researchers from Ammono, a GaN company in Poland, say they have made bulk GaN substrate material using a process that grows the GaN from a seed material in high-pressure ammonia vapor. If the technique can be fully worked out, "it would be a major enabler of progress," says James Speck, whose lab at UC Santa Barbara is working in the same area.

Meanwhile, progress in electronic applications is forging ahead on several fronts. One is the development of transistors capable of switching on and off at ultrahigh frequencies. Nitride high-frequency transistors are key to making amplifiers for satellite communications, radar devices, and cell phone base stations, Palacios says, because they are heat-tolerant, can send out large amounts of power, and can be made very small. In the March issue of *IEEE Electron Device Letters*, Palacios and colleagues report making the first-ever AlGaIn/GaN high-electron-mobility transistors that can amplify signals at a frequency of 300 billion times per second, or 300 gigahertz. Today's cell phone base stations use amplifiers that work at a rate of about 1 to 2 GHz. So if the nitrides can be made to work as reliably, they could dramatically increase the speed of wireless communications. And the progress is not likely to end soon. "We think we are far from the limit," Palacios says.

Nitride transistors might also prove essential for a variety of future "smart grid" applications. First, however, they must be able to withstand transmitting large amounts of power, something silicon-based electronics struggle with. In most nitride transistors developed so far for high-power applications, electrons can leak out of



Follow the sun. Nitride solar cells can be tailored to absorb light across the solar spectrum (*above*) and paired with silicon photovoltaics (*top*) to generate more electricity.

electrodes, causing power losses and even failure of the devices. In 2009, however, a team led by Lester Eastman, an electrical engineer at Cornell University, reported that by adding an insulating layer of hafnium dioxide to reduce leakages, they had made some of the first devices that can handle both high voltages and high current. "Before now, there were no electronic devices that could handle both high current and high voltage, but our device can do it," Eastman says. If such devices prove to be reliable, Palacios says, they could make it far cheaper to control the movement of power through the electric grid, enabling new energy production

from solar and wind and new appliances that turn themselves during off peak hours of electricity demand.

In the longer run, nitrides might also prove useful in harvesting solar energy. To capture sunlight, solar cells use the energy in photons to kick electrons up from the valence band to the conduction band. In silicon solar cells, any energy a photon has in excess of silicon's band gap turns into heat and is wasted. But researchers such as Wladek Walukiewicz, a semiconductor physicist at the Lawrence Berkeley National Laboratory in California, hope the tunable band gaps of nitrides can change that equation. In a paper published online in *Applied Physics Letters* on 11 December 2009, Walukiewicz and colleagues report pairing a GaN solar cell with a more standard silicon cell, so that the GaN cell grabbed the higher-energy photons and the silicon cell grabbed those at the lower end of the spectrum. The team didn't measure how efficiently the device converted sunlight into electricity, but Walukiewicz says such GaN and Si tandem cells could someday outperform standard silicon cells by 50%.

A final area in which the nitrides are poised to make a big impact is as highly accurate chemical sensors. Nitrides are chemically more inert than most other semiconductor sensors, says Fan Ren, a chemical engineer at the University of Florida, Gainesville, and their high electron mobility means they generate less noise and thus can spot smaller signals. That means that they can be made smaller, more sensitive, and more cheaply than other sensors. Six years ago, Ren says his team made its first nitride-based sensors to detect hydrogen fuel leaks for NASA; car dealers later adapted them to check tanks storing fuel for hydrogen-powered cars.

Ren's group and others have also tailored nitride-based transistors to detect DNA, changes in pH, and even proteins associated with breast cancer. In the January *Journal of Diabetes Science and Technology*, Ren and colleagues reported that by coating key regions of an AlGaIn-GaN transistor with a glucose-binding enzyme called glucose oxidase, they could use it to detect glucose in subjects' breath. Similar sensors might provide a noninvasive way to track a diabetic's glucose levels. Such applications have long seemed fanciful, but progress in nitride-based electronics is bringing them steadily closer.

—ROBERT F. SERVICE

PERSPECTIVE

It's Time to Reinvent the Transistor!

Thomas N. Theis* and Paul M. Solomon

A breakthrough in materials could refresh and sustain the information technology revolution.

Have you noticed that computers have stopped getting faster? Microprocessor clock frequencies plateaued around 2005, a stunning break after a decades-long run of ever-compounding improvements in computing speed. The cause is a breakdown of the simple constant-electric-field scaling rules that had guided the shrinking of field-effect transistors (FETs) for decades (1). As transistors shrank, they switched faster and used less power to switch. But constant-field scaling requires that operating voltages decrease in tandem with transistor dimensions. In recent years, this has become increasingly difficult to follow because a minimum gate voltage swing is necessary to switch the device from an "off" (low-current) state to an "on" (high-current) state. If that swing is too small, the device designer is faced with two bad choices: excessive leakage current in the nominally "off" state or low current (slow circuits) in the nominally "on" state. In other words, the choice is between computers that run hot while doing nothing or computers that run slower than previous models! Thus, over the past 10 years, the industry gradually moved from constant-field scaling toward constant-voltage scaling (2). Companies continue to shrink the transistor, emphasizing the increasing number of parallel processors (cores) they can place on a single silicon chip. But with power supply voltages stuck at about 1 V, increasing clock frequencies as in the past would result in unsupportable increases in power dissipation and heat generation. The transistor is rapidly approaching its ultimate physical limits.

The only way to decisively break the power dissipation bottleneck is to change the physics of transistor operation in ways that facilitate further reduction of operating voltage. That means increasing the nonlinearity of the switching behavior so that a much smaller swing in gate voltage is needed to switch the device from off to on. In a conventional FET, the sharpness of this turn-on is limited by the leakage current in the off state. This current arises from charge carriers in the source electrode that are thermally excited over an energy barrier controlled by the voltage on the gate

electrode and thus enter the conducting channel of the device (Fig. 1). However, there are other ways to gate the flow of carriers through the channel.

The interband tunnel FET (3) uses band-to-band tunneling to filter the energy distribution of charge carriers in the source. If electrons are the charge carriers, the injected electrons are limited in energy from below by the conduction band in the channel and from above by the valence band in the source (Fig. 2). This cuts off the hot Boltzmann tail of carriers in the source and should produce a much sharper turn-on. Promising behavior was observed in a carbon nanotube device (4). Only limited success has been obtained so far with silicon-channel devices (5). Presumably this is because band-to-band tunneling transitions in this indirect band-gap material are not allowed by momentum conservation unless a phonon is involved. Interest may therefore be shifting to direct-gap semiconductors in which it should be possible to engineer larger on-state tunneling currents (6). The best approach may be to incorporate a direct-gap material (such as a III-V compound semiconductor nanowire or a carbon nanotube) in a wirelike device geometry, which, compared with a conventional planar device structure, will allow more abrupt modulation of energy bands for increased band-to-

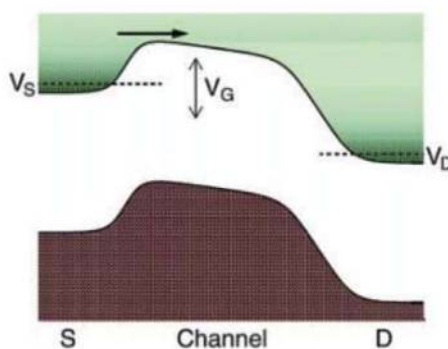


Fig. 1. The voltage V_G applied to the gate contact of a FET determines the energy barrier for injection of charge carriers from the source contact (S) into the channel. Leakage current in the off state (shown) is due to carriers in the hot tail of the equilibrium energy distribution of carriers in the source. Shade of gray indicates charge carrier concentration. D indicates the drain contact.

band tunneling and thus increased current in the on state.

Another way to increase the sharpness of the FET turn-on is to incorporate an internal gain mechanism so that a small voltage swing on the gate electrode causes a larger swing of the internal potential that gates the flow of current. Salahuddin and Datta (7) proposed a device with a gate insulator stack consisting of a layer of ferroelectric material sandwiched between conventional dielectric layers. The energy barrier presented by this gate structure to charge injection depends on the polarization of the ferroelectric layer, which, because it arises from a collective effect, switches abruptly between two polarization states. A key insight was the recognition that layer thicknesses and material properties could be engineered so as to greatly suppress the undesirable hysteretic behavior that would normally be expected from such a gate structure while still amplifying the effect of the gate potential on the channel potential. One potential drawback is that the shortest reported ferroelectric switching times, 70 to 90 ps (8), are too long to be of interest for fast FET logic, where switching can occur in 1 ps or less. But Kopp and Mannhart (9) have predicted similar effects in electronic systems exhibiting strong electron exchange and correlation effects. [See also the Review by Mannhart (10).] Regardless, Salahuddin

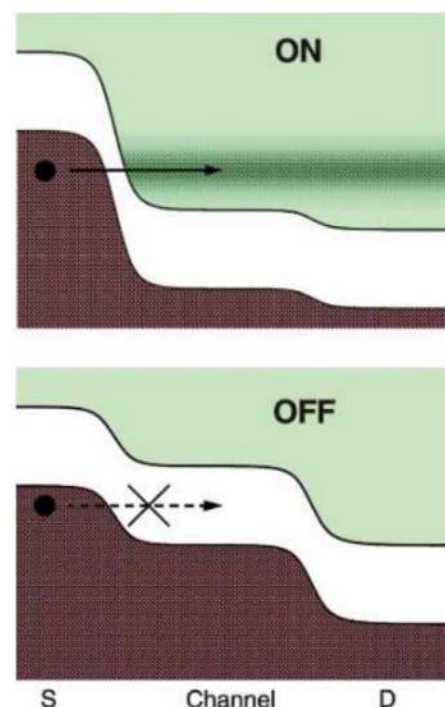


Fig. 2. Operating principle of a tunnel FET, after Appenzeller *et al.* (4). The upper (conduction) and lower (valence) energy bands are (top) crossed in the on state and (bottom) uncrossed by the gate voltage in the off state.

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and Datta have made a conceptual breakthrough by showing how a collective switching effect can be exploited to improve the nonlinearity of an FET. Further invention appears likely.

Both the tunnel-FET and the ferroelectric-gate FET promise low voltage and thus ultra-low-power switching, but as presently conceived these devices may not be fast. Can new direct-gap materials and novel device structures reduce the internal device resistance presented by the band-to-band tunneling barrier? Can the switching speed of ferroelectric materials be markedly

increased? Can the highly correlated electronic systems flagged by Kopp and Mannhart be incorporated into practical channel and gate electrode structures to produce similar but faster switching? These are great challenges for materials scientists. A breakthrough could refresh and sustain the information technology revolution for decades to come.

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PERSPECTIVE

An Emergent Change of Phase for Electronics

Hidehiko Takagi^{1,2*} and Harold Y. Hwang^{1*}

Correlated electrons in transition metal oxides can form a variety of electronic phases. The phase change between these various states gives rise to novel device functions, including sensing, signal conversion, and nonvolatile memory, and is now at the frontier of research on “emergent research device materials.” Those oxide devices may have an advantage over conventional semiconductor devices for added functionality and future downsizing to the nanoscale. The elucidation of the microscopic physics behind their operation is a key step for further development.

In the 2007 edition of the *International Technology Roadmap for Semiconductor Devices*, a new chapter was created—“Emergent Research Device Materials”—in which the needs for a new generation of devices based on novel mechanisms were emphasized (1, 2). Strongly correlated electron devices using complex oxides occupy an important position along this direction. Compared with conventional semiconductors, the quality of oxide materials for devices was believed to be substantially inferior partly because of their chemical complexity. Recent progress in oxide fabrication technology, however, is altering this conventional belief. For example, high-mobility two-dimensional (2D) electron gases were successfully formed in oxides, including (Mg,Zn)O/ZnO heterointerfaces exhibiting the quantum Hall effect (3) and delta-doped SrTiO₃ heterostructures exhibiting 2D superconductivity (4).

The electric and magnetic properties of transition-metal oxides are often dominated by electrons in *d*-orbitals. The large Coulomb repulsion be-

tween electrons accommodated in the spatially constrained *d*-orbitals tends to block the motion of electrons from one atom to another, and the electrons are highly interacting. Just like interacting atoms and molecules, the correlated electrons can form solid (insulator), liquid (metal), and superfluid (superconductor) states inside the solid. The presence of the electron's three degrees of freedom—charge, spin, and orbital—enrich these electronic phases further (Fig. 1A). Complex combinations of charge-, spin-, and orbital-ordered states indeed appear in the phase diagrams of transition metal oxides.

These rich electronic phases compete with each other in a delicate balance. Even a minute external perturbation by an applied electric or magnetic field or pressure can induce a phase change (5), giving rise to a dramatic response to external stimuli. The coexistence of charge and spin degrees of freedom can bring about cross-couplings between the electric and magnetic response. This coupling is the hallmark of phase-change functions in transition metal oxides, rendering them useful as sensors and memories and for signal conversion. Well-known examples of this approach are so-called “colossal magnetoresistance” (CMR) devices that use complex Mn oxides for magnetic field sen-

sors (6). Large regions of their phase diagrams show a critical competition between a ferromagnetic metal (liquid) and a charge-ordered insulator (solid). In such a situation, the electron solid is melted easily by the application of magnetic fields, and the ferromagnetic metal state is stabilized, which manifests itself as an orders-of-magnitude change in resistivity. Melting of the electron solid also occurs by the irradiation of pulsed photons (7), which can form the basis of an optical switch. Multiferroics, in which ferromagnetism (or antiferromagnetism) coexists with ferroelectricity, falls into the same category of phase-change function (8). The critical control of the spin state via magnetic field allows the control of electric polarization, including its orientation.

Control of magnetization via electric field is now envisaged (9), which should affect applications in spintronics, in which the spin of the electron in addition to its charge is used to convey information. A critical electronic phase change, if coupled with a chemical phase change, produces a nonvolatile memory effect. With demand for increased storage capacity, modern nonvolatile memory based on semiconductors, such as flash memory, has been downscaled successfully but is expected to soon reach fundamental physical limits. To achieve further storage density, a number of novel random access memories (RAMs) have been studied, including FeRAM (ferroelectric), MRAM (magnetic), and PRAM (phase change). ReRAM (resistance), which is based on transition metal oxides, recently emerged as a new memory candidate (10). The device has a capacitor-like structure with a metal/oxide insulator/metal (MIM) stack and, after the first electric field stress (the forming process), switches between high- and low-resistance states with the application of short voltage pulses.

Such resistance-switching phenomena had been observed in MIM structures many decades ago (11). This gained renewed interest because of the demonstration of a MIM RAM device that uses charge-ordered (Pr,Ca)MnO₃ (12) and Mott insulating NiO (13). In several cases, the

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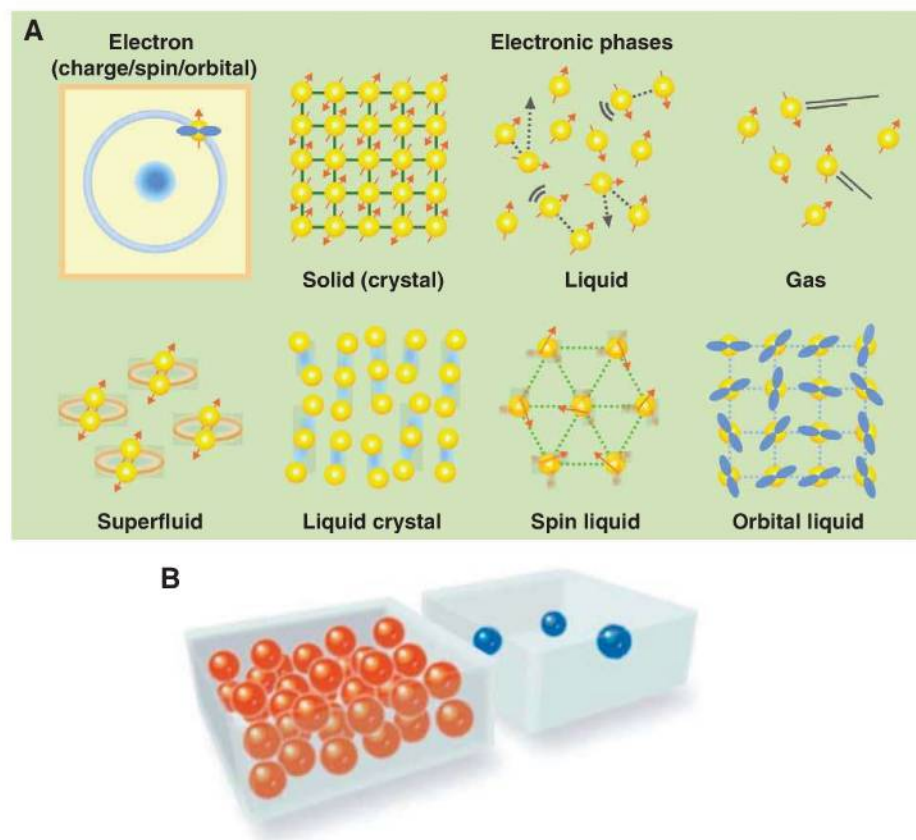


Fig. 1. All together now. (A) Correlated electrons in transition metal oxides form a variety of electronic phases. The spin, charge, and orbital degrees of freedom often behave as if they were independent of one another, which gives rise to a wide diversity of phases, including liquid states of spins and orbitals. (B) At the nanoscale, conventional devices based on semiconductors suffer from the extremely small number of carriers within a device and large phase fluctuations. This serious constraint can be resolved in devices that are based on (correlated) transition metal oxides because of the orders-of-magnitude-greater carrier density.

local reduction/oxidation of a conducting filament created by the forming process induces an electronic phase change, and hence memory operation. In this sense, ReRAM may fall into the same category as PRAM. Prototype devices have shown switching within 10 ns (14). The simple structure enables device scaling, and devices with dimensions of less than 100 nm have been demonstrated (15).

Further opportunities to reduce the size to nanometer-scale oxide devices have been demonstrated through the use of phase changes induced by scanning probes. The conducting interface between LaAlO_3 and SrTiO_3 (16), dislocation cores in SrTiO_3 (17), and ultrathin ferroelectric films (18) have all been shown to reliably switch on scales below 10 nm. Some of these examples suggest that in addition to improving materials perfection, defect engineering can also provide important opportunities for nanoscale devices.

The electronic phase change provides a variety of novel functions beyond conventional semiconductor devices, including entropic and

mechanical functions. As a consequence of their multiple degrees of freedom, some electronic phases are highly entropic, and in fact the change of volume enthalpy associated with an electronic phase transition is sometimes comparable with that of the water-ice transition (19). Applications of such large enthalpy include “electronic” ice packs, thermoelectric devices (20), and magnetic/electric refrigeration (21). Other functions are produced by the electron-lattice coupling, including giant negative thermal expansion (22) and magnetostriction. Multiferroics also may fall into this category.

Because of the presence of a rich variety of competing electronic phases, devices based on correlated transition metal oxides emerge as a rich playground for development. They have in principle advantages in device scaling because correlated oxides have essentially metallic electron densities (10^{22} to 10^{23} cm^{-3}), even in their insulating phases (23) (Fig. 1B). This should help ensure a sufficient number of carriers in a nanoscale device to avoid the limits of density fluctuations, which are becoming in-

creasingly important in conventional semiconductor devices. However, partly because of the complexity of the many-body physics behind it, it is hard to design a correlated electron device from first principles or the equivalent of semiconductor band diagrams. We believe this is a central challenge for correlated electron physics.

The basic issues to be resolved include the impurity and interfacial states formed in correlated insulators and metals, in which many-body effects may require qualitatively distinct physics. Indeed, it should be acknowledged that in many of the recent device examples discussed here, the role of electron correlations is unclear or absent. Closely related with this, we do not yet know down to what length-scale electronic phases and their transitions can be reasonably defined. Recent work using scanning tunneling microscopy indicates the presence of strong phase variations over a few nanometers (24). This is enticing in showing that a pathway to ultra-small correlated oxide devices based on phase changes exists. It also demonstrates a fundamental need to further develop our quantitative understanding of strongly correlated transition metal oxides in general, as well as their engineering in device form.

References and Notes

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REVIEW

Materials and Mechanics for Stretchable Electronics

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Recent advances in mechanics and materials provide routes to integrated circuits that can offer the electrical properties of conventional, rigid wafer-based technologies but with the ability to be stretched, compressed, twisted, bent, and deformed into arbitrary shapes. Inorganic and organic electronic materials in microstructured and nanostructured forms, intimately integrated with elastomeric substrates, offer particularly attractive characteristics, with realistic pathways to sophisticated embodiments. Here, we review these strategies and describe applications of them in systems ranging from electronic eyeball cameras to deformable light-emitting displays. We conclude with some perspectives on routes to commercialization, new device opportunities, and remaining challenges for research.

Biology is soft, elastic, and curved; silicon wafers are not. An electronics technology that overcomes this fundamental mismatch in mechanics and form will enable applications that are impossible to achieve with hard, planar integrated circuits that exist today. Examples range from surgical and diagnostic implements that naturally integrate with the human body to provide advanced therapeutic capabilities, to cameras that use biologically inspired designs to achieve superior performance. Sensory skins for robotics, structural health monitors, wearable communication devices, and other systems that require lightweight, rugged construction in thin, conformal formats will also be possible. Establishing the foundations for this future in electronics represents an emerging direction for research, much different from the one dictated by the ongoing push toward smaller and faster devices that are still confined to the planar surfaces of silicon wafers.

Work toward mechanically unconventional forms of electronics began, in earnest, ~15 years ago with polymer transistors formed on bendable sheets of plastic (1, 2), where paperlike displays (3, 4) represented the main target application. Advances in printing and related patterning techniques (5) and in organic semiconductors (6) were key to much of the initial progress in this field. Research in the past few years on ultrathin inorganics, nanotubes, and nanowires promises fur-

ther improvements, as described in recent review articles (7–9). Many successes, in the form of working demonstration devices with hundreds to thousands of active components, have already been achieved; several, including displays, are nearing commercial reality.

More recently, the scope of research has expanded dramatically to include more compelling, and more technically challenging, opportunities in soft, biointegrated devices; in curved, bioinspired

designs; and in other areas. Here, the electronics must not only bend but also stretch, compress, twist, and deform into complex, curvilinear shapes while maintaining levels of performance, reliability, and integration that approach those of well-developed wafer-based systems. Stretchable electronics can be achieved in two conceptually different, but complementary, ways. One relies on the use of new structural layouts in conventional materials (10), the other on new materials in conventional layouts (11). We focus first on approaches that have yielded the most sophisticated devices and then present a more comprehensive summary of options.

Structures That Stretch

Two simple ideas underlie the strategy based on structure. The first exploits an elementary result in mechanics: Any material in sufficiently thin form is flexible, by virtue of bending strains that decrease linearly with thickness. A silicon wafer is brittle and rigid, but nanoscale ribbons, wires, or membranes of silicon are flexible. For example, ribbons with thicknesses of 100 nm experience peak strains of only 0.0005% upon bending to radii of curvature of 1 cm. Even when mounted on sheets of plastic with thicknesses of 20 μm , the strains (~0.1%) at similar bend radii remain well below the fracture limits (~1%) (12). Circumstances can be improved further by moving the silicon away from the surface of the

plastic, where the bending strains are largest, to the point in its depth where these strains are zero. Ultrathin circuits that use silicon nanoribbons in this type of neutral mechanical plane design can be bent to radii of ~150 μm , with strains in the silicon that are less than ~0.1% (12, 13).

Configuring such structures into “wavy” shapes and bonding them to elastomeric substrates yields systems that can not only flex but also stretch and compress, with a mechanics similar to that of an accordion bellows. Figure 1, A and B, shows two possibilities, illustrated with ultrathin sheets of silicon integrated on slabs of the elastomer poly(dimethylsiloxane) (PDMS). The first case (Fig. 1A) corresponds to a sheet formatted into waves with a herringbone configuration by a controlled buckling process (14), as a two-dimensional analog of a similar effect first observed in ribbons (15). The resulting Si/PDMS construct can be stretched and compressed reversibly, with a linear elastic response to applied force. The amplitudes and wavelengths of the waves change in response to induced deformations

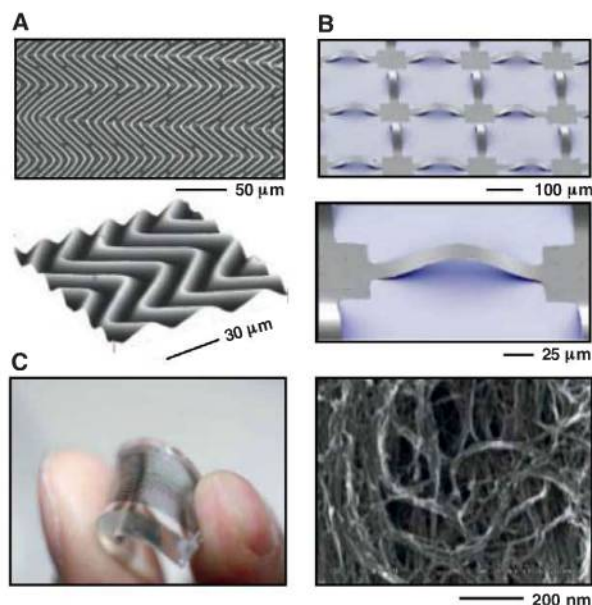


Fig. 1. Concepts for stretchable electronic materials. (A) Stretchable silicon membrane (~100-nm thickness) configured in a wavy shape and bonded to a piece of rubber, presented in optical (top) and atomic force (bottom) microscope images. (B) Extremely stretchable silicon membrane (~100-nm thickness) patterned into a mesh geometry and bonded to a rubber substrate only at square pads located between arch-shaped bridge structures, presented in moderate (top) and high (bottom) magnification scanning electron microscope (SEM) images. The PDMS is colorized blue. (C) Stretchable conductive lines of a SWNT gel printed on a slab of rubber (left) and SEM of the networks of SWNTs that provide electrical pathways in these composites.

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in a way that involves considerable strains in the PDMS, but not in the silicon. In particular, mechanics modeling reveals that the peak strains in the silicon can be 10 to 20 times as small as the applied strains (16). Such designs represent, then, a stretchable form of silicon with a strain range of 10 to 20%, that is, 10 to 20 times as large as the intrinsic fracture limits of the silicon.

A related strategy improves this range by structuring the sheet into a mesh and bonding it to the PDMS only at the nodes. The buckled, arc-shaped interconnecting structures (Fig. 1B) can move freely out of the plane to accommodate applied strains of 100% or more, even to values that approach the fracture limits of the PDMS (17). Related approaches that use mesh layouts with planar leaf-arm (18, 19), coiled spring (20), and noncoplanar serpentine (17) interconnects also have attractive features. Alternative, simpler designs (21) provide stretchability in certain directions; they, like the arc-shaped layouts of Fig. 1B and related serpentine configurations (17), have been used to achieve integrated systems, as discussed in a following section.

Materials That Stretch

New materials provide an alternative route to stretchable electronics. The most successful approaches use elastic conductors as electrical interconnects between active devices that are rigid or only bendable. Although conductive rubbers based on elastomers loaded with carbon black have been known for decades, the resistances and their dependence on strain are both too large to be useful. In a much more promising and recent approach, long, single-walled carbon nanotubes (SWNTs) serve as conductive dopants in a rubber matrix (22, 23). Here, SWNTs processed by grinding in an ionic liquid and then mixing with a fluorinated copolymer yield a black, pasty conductive substance, referred to as a bucky gel (22, 23). Individual SWNTs form tangled mats in these gels, with the capacity to reconfigure in response to applied strain in a manner that preserves highly conductive pathways for charge transport. This material can be printed onto sheets of PDMS to yield elastic conducting traces with stretchability in the range of 100%. Figure 1C shows a picture of such a sample and a micrograph of the associated network of SWNTs. Alternative, related approaches use SWNTs in thin

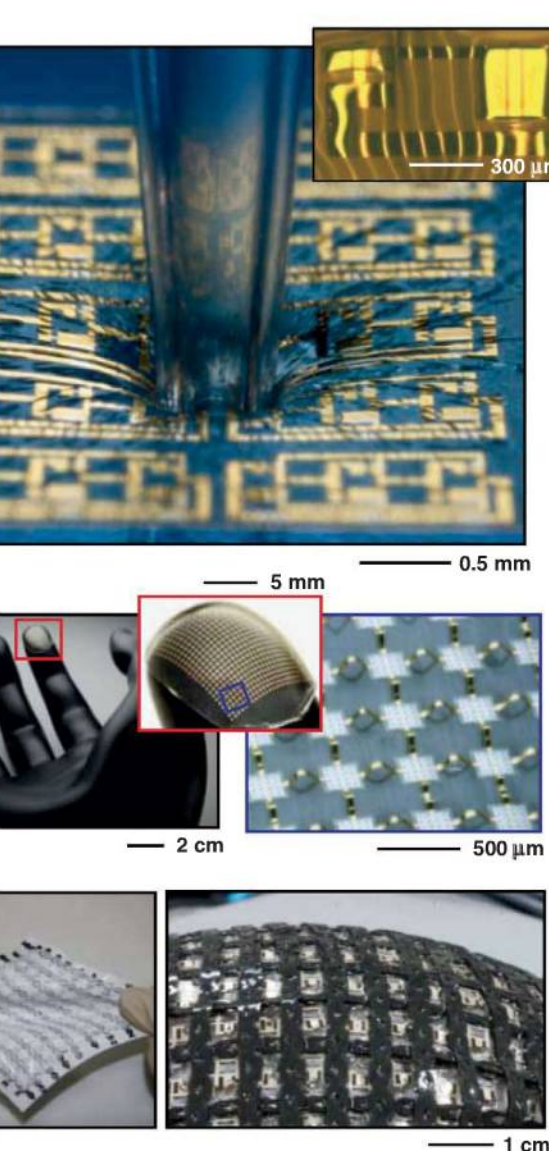


Fig. 2. Examples of stretchable electronics. (A) Stretchable silicon circuit in a wavy geometry, compressed in its center by a glass capillary tube (main) and wavy logic gate built with two transistors (top right inset). (B) Stretchable silicon circuit with a mesh design, wrapped onto a model of a fingertip, shown at low (left), moderate (center) and high (right) magnification. The red (left) and blue (center) boxes indicate the regions of magnified views in the center and right, respectively. The image on the right was collected with an automated camera system that combines images at different focal depths to achieve a large depth of field. (C) Array of organic transistors interconnected by elastic conductors on a sheet of PDMS in a stretched (left) and curvilinear (right) configuration.

film networks formed by solution casting or other means (24–26).

Stretchable Electronics, Optoelectronics, and Integrated Systems

The ideas of Fig. 1 can be exploited to yield stretchable, integrated systems. Figure 2A presents an example of the design approach of Fig. 1A applied to an ultrathin, neutral mechanical circuit sheet that supports an array of silicon transistors, logic gates, and ring oscillators (13). A pair of transistors in an

inverter appears in the upper inset. The waves are influenced by variations in thicknesses and material compositions across the area of the circuit to yield layouts that are much more complex than those in Fig. 1A. The mechanical responses that enable stretchability, however, are the same: The wavy shapes change to accommodate applied strains, and the underlying PDMS substrate provides an elastic restoring force. The main image shows the circuit deformed in its center with a glass pipette to illustrate the “soft,” elastic nature of this system. Figure 2B presents an example with a layout similar to that of Fig. 1B. Here, microscale arc-shaped “ribbon cables” of metal and plastic in neutral mechanical layouts interconnect silicon devices located at the nodes of the mesh (17). The circuit is conformally integrated onto a model of a fingertip (27), as an example of a surface whose nonzero Gaussian curvature would be impossible to wrap with a system that is only flexible. Figure 2C shows a similar outcome achieved with stretchable conductors (22). This circuit consists of arrays of organic transistors interconnected by printed bucky gels (Fig. 1C) and supported by a thin sheet of PDMS in stretched and curvilinear configurations. During deformation, only the interconnection lines stretch, such that negligible changes in transistor characteristics occur even for strains up to 70%.

The left frame of Fig. 3A shows an image of an integrated system that exploits the concepts of Fig. 1B and Fig. 2B, that is, a digital camera based on an array of silicon photodetectors in the approximate size and curved layout of the human retina (28). This design offers enhanced field of view and uniformity in illumination compared to a comparable planar detector, when simple imaging optics are used (28, 29, 19). Fabrication of such an “eyeball” camera starts with an array of silicon photodiodes and blocking diodes formed in a planar but stretchable configuration. Conformal wrapping onto a concave, hemispherical glass substrate, followed by integration with an imaging lens and a printed circuit board interface to a computer for data acquisition, completes the device. A picture of an eye, captured with a camera whose detector curvature (i.e., elliptical paraboloid) matches the image surface formed with a plano-convex lens, appears on the right in Fig. 3A (29). The top and bottom frames correspond to the

image rendered in the curved format of the camera and a planar projection, respectively; the inset illustrates the picture that was imaged. These ideas create new engineering options in imaging devices, where the geometry of the detector array can be optimized together with the lens configuration. The most promising initial application opportunities are in surveillance, night vision, and endoscopy, where considerations of cost, size, and/or weight can be addressed by introducing curvature in the detector to enable dramatic reductions in the complexity of the optics. The same concepts have the potential to allow other, more complex biologically inspired designs. Ultimately, such devices might be used as retinal implants or as active components on the eye to restore or enhance vision.

Light-emitting devices are also possible using the same approaches. Figure 3B shows an image of a stretchable inorganic light-emitting diode (LED) display that uses ultrathin, microscale AlInGaP LEDs interconnected in a mesh layout and bonded to a PDMS substrate (30). Figure 3C provides an example of a related demonstrator produced with stretchable conductors and organic LEDs (23). Both types of display can be stretched by 30 to 50% and wrapped onto curvilinear supports without any mechanical damage or change in operating characteristics. This class of technol-

ogy could, of course, be useful as a flexible lighting or display system, with extreme levels of bendability and mechanical robustness. More interesting opportunities in the future might lie in optogenetics, where programmable light sources wrapped onto the convoluted surface of the brain could provide insights into brain function. Light-based therapies and diagnostics in other parts of the body might also be achievable in similar tissue-integrated modes.

Stretchable Electrodes

Work in stretchable electrodes, as opposed to electronics, has a comparatively long history and broad range of materials and design options. In fact, the field of stretchable electronics owes its origins to observations that films of gold formed by physical vapor deposition directly onto PDMS spontaneously adopt microstructured or nanostructured forms (31) and that these structures (Fig. 4A) provide electrodes that can accommodate large applied strains without fracture (32). Detailed studies suggest that stretchability in this case derives from a physics similar to that of the silicon structures of Fig. 1A but with additional contributions from the motion of microscopic cracks that form in the films during fabrication and subsequent deformation (33). Figure 4B shows a different, but related, ex-

ample in which direct writing with a silver nanoparticle ink yields a wavy metallic microwire (34), able to accommodate stretching through changes in wavelength and amplitude. Similar one-dimensional wavy shapes can be achieved in conducting and semiconducting nanomaterials. Figure 4C presents atomic force microscope measurements on an individual SWNT (35), formed in a wavy configuration on PDMS using the techniques that yielded the structure of Fig. 1A. Newtonian mechanics models like those that describe similar deformations in silicon nanoribbons (15) can capture the physics even at the molecular scale of the nanotube (35). With nanowires, related types of wavy deformations form in the plane of the substrate. Lithography can yield similar structures in micron-scale metal wires, as illustrated with a stretchable, high-frequency interconnect shown in Fig. 4D (36). Such traces can serve as interconnects between integrated circuit chips as a route to stretchable devices (37) with a type of spatially discrete functionality that can complement the capabilities of the distributed systems of Figs. 2 and 3.

Finally, in addition to structured wires of Fig. 4, A to D, similar levels of stretchability can be achieved directly in electrodes of suitable materials, like the SWNT-based conductors of Fig. 1C. Figure 4E shows, as an example, a low-melting-point metal solder housed in sealed microchannels in PDMS (38). Here, the metal can plastically deform and flow in response to large strain deformations, while the PDMS provides an elastic restoring force. More recently, work shows that graphene on PDMS exhibits reversible responses to large applied strain (39), perhaps involving a mechanics similar to that of the gold electrodes of Fig. 4A. An image appears in Fig. 4E.

Paths to Commercialization

Although nearly all current activities in stretchable electronics are centered in academic laboratories, there is growing interest at small and large companies. A path to commercialization might begin with stretchable electrodes, followed by devices with discrete configurations, and culminating in highly functional, distributed systems. An important perspective is that many of the underlying concepts are aligned well with the incremental, but collectively substantial, developments in silicon packaging. Three examples are noteworthy. First, the commercial success of ultrathin cell phones and laptop computers with hinged displays, sliding keyboards, and related components continues to motivate the development of sophisticated, multilayer flexible interconnection cables and printed circuit boards that can accommodate bending to small radii of curvature. Second, technology for thinning silicon chips to thicknesses in the range of tens of microns is of increasing importance for advanced thermal management and three-dimensional, stacked integration schemes. A third effort in industry seeks to evolve chips into forms that seamlessly integrate with printed circuit boards, in a manner that blurs

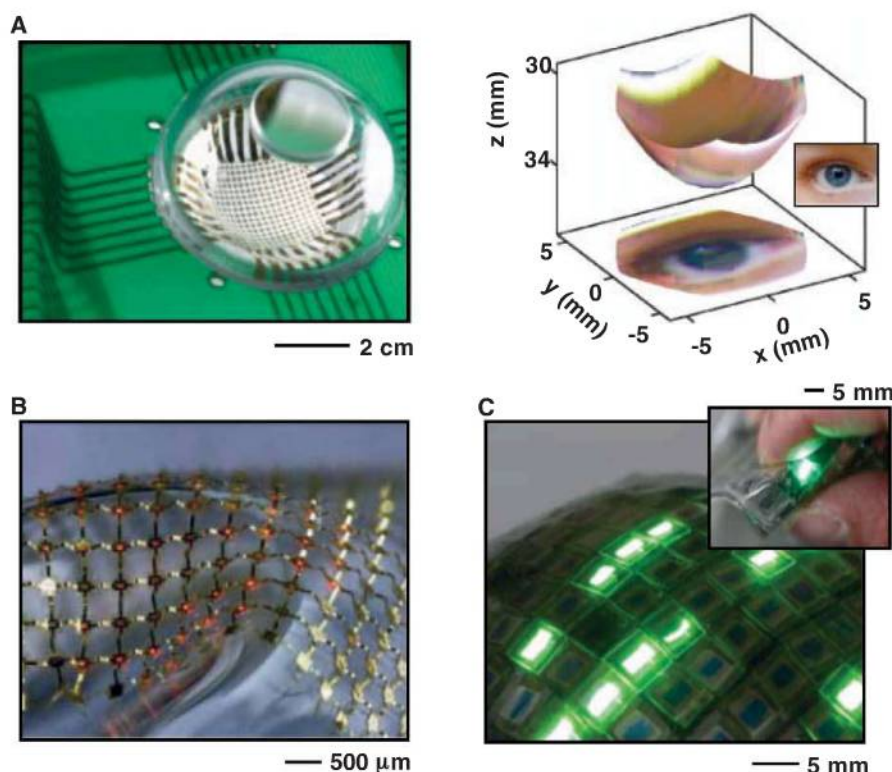


Fig. 3. Examples of stretchable electronic systems. (A) Electronic eyeball camera (left) that uses a hemispherically curved array of silicon photodetectors and picture collected with a similar camera that uses a paraboloid design (right). The image in the top is rendered in a form consistent with the curvature of the detector. A planar projection appears on the bottom, with the actual object in the center right inset. (B and C) Stretchable LED display devices that use mesh designs with microscale inorganic LEDs (B) and stretchable interconnects with organic LEDs (C).

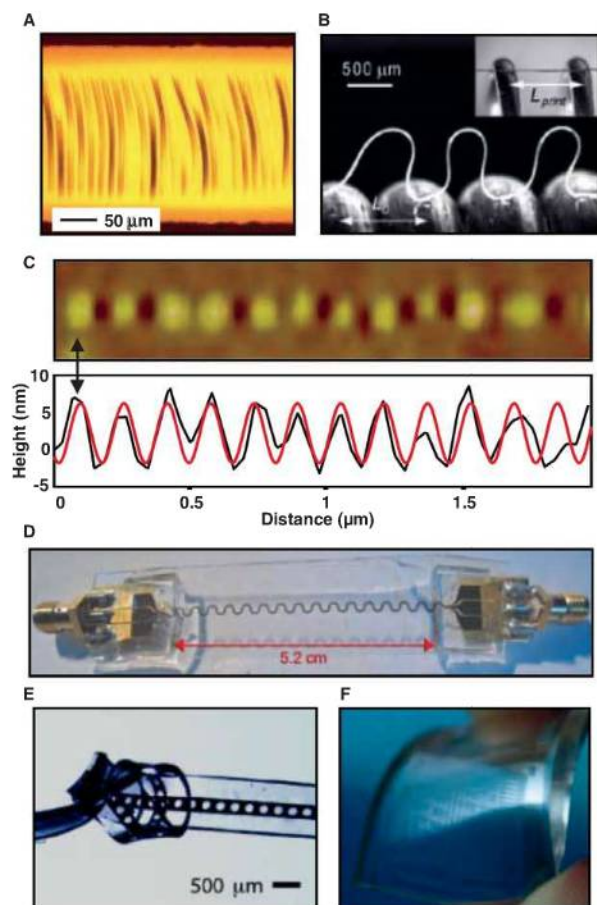


Fig. 4. Structures and materials for stretchable electrodes. (A) Thin gold film formed by physical vapor deposition onto a PDMS substrate, with stretchability provided by wavy shapes and networks of micro- and nanocracks. (B) Wavy silver microwire formed by direct write printing onto the surface of a metal spring. The inset shows the structure in its original configuration. (C) Atomic force micrograph (top) and surface relief profile (bottom; black: data; red: sinusoidal fit) of an individual SWNT formed into a wavy shape by transfer to a prestrained substrate of PDMS followed by release. (D) Serpentine metal coplanar waveguide embedded in a slab of PDMS and attached to standard SMA connectors for a stretchable, high-frequency cable. (E) Stretchable metal wire consisting of a low-melting-temperature solder sealed in channels in PDMS. (F) Patterned layer of graphene on a PDMS substrate.

the distinction between the two. Interest in such configurations is motivated by their ability to eliminate parasitic effects that reduce performance. The design and manufacturing strategies associated with these and other trends in packaging are highly relevant to certain approaches in stretchable electronics, and the reverse is also true. This situation creates promising paths to commercialization, with time scales that have the potential to be much shorter than those typically associated with new technologies derived from academic research.

Challenges and Outlook

Collectively, the advances in materials and mechanics described here provide several promising engineering options for stretchable, curvilinear electronic and optoelectronic components and complete,

integrated systems in discrete, distributed, or hybrid forms. The underlying science encompasses many research topics of fundamental interest, from materials growth, processing, and heterogeneous integration, to micro-mechanics and nanomechanics, to charge transport and its coupling to strain and geometry, to adhesion and interface science. To appreciate how some of these topics relate to engineering challenges, consider that the modulus of silicon is $\sim 100,000$ times as high as a typical elastomer; the thermal conductivity is ~ 1000 times as great, and the thermal expansion coefficient is ~ 100 times as small. Such extreme mismatches in properties lead to interesting, and similarly extreme, behavior in systems that intimately integrate these dissimilar materials. As an example, research on nonlinear behavior in hard and soft laminates is providing new insights into the mechanics of their deformation (16, 33, 40, 41), particularly their nonlinear behavior in buckling modes, with explicit relevance to stretchable electronics. The challenges are even more pronounced for SWNTs (22–26) and graphene (39), where larger mismatches in properties occur and little is known about even the basic foundational mechanics responsible for stretchability. Other areas for study in these and other heterogeneous systems include the physics of heat transport, to enable efficient thermal management, and the materials science of interfaces, to

ensure mechanical reliability. From an engineering standpoint, these and related issues must be understood clearly before levels of integration in stretchable electronics, which currently range, in distributed forms, from hundreds to thousands of transistors, can begin to reach those of established devices.

Successful outcomes from these efforts have the potential to change fundamentally our conception of electronics, from hard, rigid, planar chips to soft, stretchable, curvilinear sheets. Here, mechanics design will be as important as electrical design in the formulation of new systems. Of the many potential areas of application, some of the most compelling are in biomedical devices that address important problems in human health. Soft, elastic mechanical properties and curvilinear layouts can provide both

mechanical modulus and shape matching to biological tissues. Such “tissue-like” devices, particularly when implemented with biocompatible materials, will facilitate solutions to long-standing challenges in the establishment of viable, intimate biotic-abiotic interfaces with high levels of functionality. Recent work in this direction using passive, stretchable electrodes for research applications (42) and diagnostic systems with discrete designs (37), both with modest degrees of deformability, will rapidly expand into fully distributed, biointegrated, multi-functional devices for clinical use.

Beyond biology, stretchable electronics will find utility in conformal, active antennas and other components for communications, perhaps even in cellular telephones of the future, as envisioned recently by a large company in this industry. In other possibilities, stretchable sensor tapes will provide thin, conformal monitors of the structural health in the wings of aircraft or the blades of windmills, as examples. The basic ideas can also be exploited in other semiconductor technologies, including photovoltaics and thermoelectrics. In the latter case, devices for scavenging power will be available in the form of sheets that can wrap the complex surfaces of engine parts for efficient thermal coupling. These and related opportunities for engineering in areas of application with important societal implications, taken together with the broad range of interesting scientific topics, provide strong motivation for continued and expanded efforts in this emerging field.

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REVIEW

Oxide Interfaces—An Opportunity for Electronics

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Extraordinary electron systems can be generated at well-defined interfaces between complex oxides. In recent years, progress has been achieved in exploring and making use of the fundamental properties of such interfaces, and it has become clear that these electron systems offer the potential for possible future devices. We trace the state of the art of this emerging field of electronics and discuss some of the challenges and pitfalls that may lie ahead.

Herbert Kroemer began his Nobel lecture by stating, “Often, it may be said that the interface is the device” (1). Transistors, lasers, and solar cells all exploit interfacial phenomena. Interfaces enable data processing, memory, and electronic communication. Moreover, interfaces in semiconductor structures are the birthplace of a multitude of fascinating discoveries in fundamental science. Curiously, away from interfaces, in the bulk of the material, the behavior of electrons in semiconductors such as silicon is less exciting. The electrons zip through the crystal lattice essentially as independent, free particles, barely interacting with one another. In contrast, there are other materials (e.g., many oxides) in which electron interactions in the bulk of the material give rise to spectacular phenomena, including colossal magnetoresistance and high-temperature superconductivity.

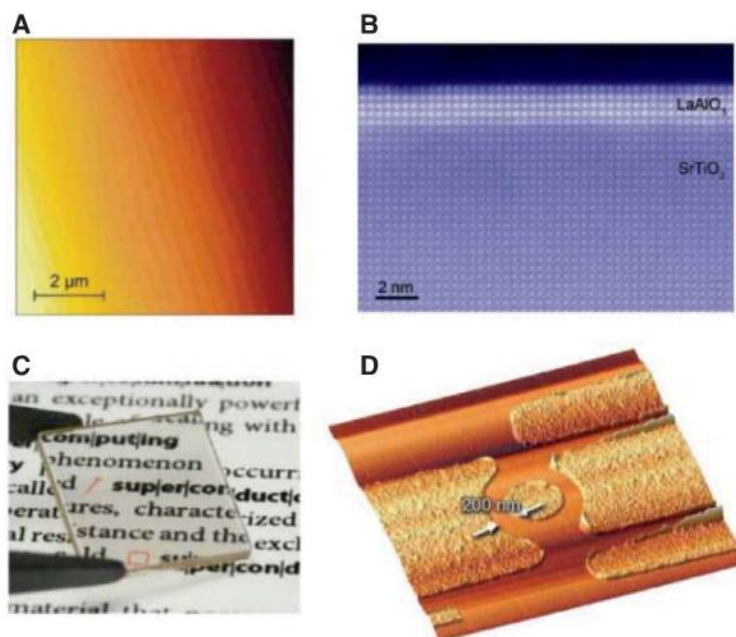


Fig. 1. Micrographs of $\text{LaAlO}_3\text{-SrTiO}_3$ heterostructures. (A) Top view of a $\text{LaAlO}_3\text{-SrTiO}_3$ bilayer containing eight monolayers of LaAlO_3 , taken by scanning force microscopy (figure courtesy of S. Paetel). (B) Cross-sectional view of a corresponding sample containing five monolayers of LaAlO_3 (figure courtesy of L. Fitting Kourkoutis and D. A. Muller). (C) Optical photograph of a complete sample (figure courtesy of G. Hammerl and K. Wiedenmann). (D) Scanning force microscopy image of a conducting ring patterned by electron beam lithography into a $\text{LaAlO}_3\text{-SrTiO}_3$ structure [from (8)].

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These phenomena arise in oxides from regularly spaced ions interacting with the electrons, from the unique electronic character of oxygen ions, and from the electronic correlations—interactions among the electrons, which make them deviate from free-particle behavior.

Interfaces Matter

The introduction of interfaces into semiconductor structures spawned numerous semiconductor devices of immense utility and interesting physics. By analogy, it is tantalizing to incorporate well-defined interfaces into oxides to generate novel phenomena. Because oxide multilayers are far more difficult to grow than semiconductor heterostructures, progress in this direction was thwarted for many years. Intensive efforts over the past two decades to grow oxide superconductors, however, led to decisive progress in the growth of oxide multilayers (Fig. 1). Key steps were the ability to terminate oxide substrates at well-defined ionic planes (2), the application of pulsed-laser deposition (PLD) (3) and molecular-beam epitaxy (MBE) (4) to the growth of multicomponent oxides containing difficult-to-oxidize constituents, and the development of high-pressure reflection high-energy electron diffraction (5) to monitor the deposition of individual atomic layers. As a result, epitaxial heterostructures of oxides can now be grown with atomic-layer precision. The chemical abruptness and crystalline perfection of oxide multilayers now rival those of semiconductor multilayers; it is possible to change from one material to another over a distance of a single unit cell (6, 7) (Fig. 1B). Such oxide heterostructures can also be patterned laterally (8) (Fig. 1D), even with nanometer resolution (9). The ability to precisely create interfaces connecting different oxide materials provides a wealth of new possibilities to generate novel electronic phases.

The same phenomena that control interfaces in standard semiconductors, such as the formation of space charge layers, are also relevant at oxide interfaces. Mobilities of charge carriers have reached values so high that the quantum Hall effect (QHE) has been achieved at interfaces between oxides, an effect previously limited to interfaces between high-purity semiconductors and to interfaces involving graphene sheets. As a result of improvements in film growth, the mobility of two-dimensional elec-

tron gases (2DEGs) at ZnO-Mg_xZn_{1-x}O interfaces has now surpassed 20,000 cm² V⁻¹ s⁻¹ (0.5 K) (10). The QHE found in ZnO-Mg_xZn_{1-x}O (Fig. 2) closely resembles the QHE of GaAs-Al_xGa_{1-x}As interfaces because ZnO is, like GaAs, devoid of strong electronic correlations [Zn²⁺ in ZnO has a full shell electron configuration (3d¹⁰)]. Strong correlations may be added, however, by altering the material—for example, by incorporating monolayers with strong electronic correlations close to the interface. An exciting prospect for future research is to find out which phases the 2DEGs will generate if, for example, the QHE and fractional QHE states are coupled to superconducting or magnetic states.

The physical phenomena possible at oxide interfaces go well beyond those exhibited by conventional semiconductor interfaces because new and largely unexplored physics becomes relevant, enabling novel electronic phases. The key factors controlling the electronic behavior that can arise at precisely tailored interfaces between transition metal oxides, an important class of materials for emerging electronics, are described next.

Ionicity and Oxygen

Because of the strongly ionic character of transition metal oxides, the Coulomb interaction of an ion with the host lattice, the Madelung energy, plays an important role in determining interface properties. The Madelung energies of interface ions differ appreciably from those of the same ions well away from the interface in the bulk of the material, altering the energies of electronic states in the vicinity of the interface. Moreover, as a result of the ionic character of the lattice, electronic orbitals in oxides tend to overlap less than the hybridized s- and p-orbitals characteristic of standard semiconductors. The characteristic bandwidths of transition metal oxides are therefore

smaller than those of conventional semiconductors. As a result, the effective masses of the charge carriers in oxides exceed those of electrons in conventional semiconductors by an order of magnitude or more; the carriers move more slowly, thereby coupling more strongly to (slow) polarization effects of the lattice, and are more localized.

The energies by which bands bend at interfaces involving transition metal oxides are thus comparable to the bandwidths of the oxides themselves. This enables built-in potentials at interfaces to shift the Fermi energy through and beyond complete bands, causing phase transitions or otherwise markedly altering the electronic properties. By the same token, the Fermi energy of planes parallel and close to the oxide interface (e.g., the planes one and two unit cells away from the interface) may reside at rather different levels with respect to the band edges. Therefore, in different planes the electronic states at the Fermi energy may, for example, originate from different atomic orbitals, causing such planes to have disparate electronic characters and to be only weakly coupled with each other, even though these planes are all chemically identical.

Many oxide interfaces become electronically active by altering the hybridization of the ionic orbitals and by modifying the orbital and spin ordering that can occur in oxides and are coupled to the lattice structure. The d-orbital lobes of the transition metals, for example, tend to align with the crystallographic axes. Orbital and spin ordering may be frustrated or even generated at interfaces. Such effects are again unknown in conventional semiconductors.

The unique character of oxygen ions is another key factor in generating novel electronic properties at oxide interfaces [see, e.g., (11)]. O²⁻ ions are exquisitely polarizable, typically causing a large, nonlinear, and nonuniform polarizability of the lattice. Because the carrier density at oxide interfaces is high (~10¹³ to 10¹⁴/cm², as opposed to the 10¹¹ to 10¹²/cm² found in semiconductor heterostructures), this polarizability often leads to electrostatic screening lengths in complex oxides of 1 to 100 nm, which are smaller than typical screening lengths in semiconductors, again giving rise to more local, more confined novel interfacial properties. Oxygen is also special because holes in the oxygen 2p band give rise to magnetism, which, for example, is predicted to occur at the step edges of NiO surfaces (12).

Electronic Correlations

The most exciting phenomena arising at or awaiting discovery at customized oxide interfaces result from correlated electron effects. In correlated electron systems, the interaction of an electron with other electrons can no longer be described by the Coulomb interaction of a virtually free, independent particle with an average background charge that represents the other electrons. In a highly complex, nonlinear manner, electronic correlations determine the phases an electron system possesses, and thereby its characteristic properties. The strengths and relevance of the correlations

among electrons are characterized by parameters such as the on-site Coulomb repulsion energy U and exchange energies J . As these parameters are sensitive to the local ionic structure, they are usually highly susceptible to change at interfaces (13). One example is an interface-driven reduction of the electronic screening, possibly caused by the broken periodicity of the ion lattice, leading to an enhanced on-site Coulomb energy U .

Figure 3 illustrates this key issue by sketching the electronic systems at interfaces between conventional semiconductors and between correlated materials. Semiconductor interfaces are characterized by band bending and space charge layers. At such interfaces the semiconductors' carrier densities n are altered, as drawn for an exemplary case in Fig. 3A. In contrast, the electronic system of a material with correlated electrons may show several electronic phases already in its bulk (Fig. 3B, bulk phase diagram at the left). At the interface, space charge layers are induced and the carrier density changes. The interface may consequently undergo an "electronic reconstruction" (14) in which the resulting electronic phases now correspond to those of the bulk at the altered carrier density. As a result, the single-electron picture used to construct band diagrams at semiconductor interfaces collapses. It fails by not including electronic reconstruction, a key aspect of the physics of interfaces in correlated materials.

The difference between interfaces involving strong electronic correlations and semiconductor interfaces can, however, be even more striking. At interfaces in strongly correlated systems, the correlation parameters may be changed to values that are unachievable in the bulk. Because electron correlation parameters underlie all electronic properties, completely novel electronic systems with unique electronic properties can thus be generated at interfaces (15–18) (Fig. 3C). Correlated electron systems may even emerge at interfaces between uncorrelated host materials. Although experimental evidence for the emergence of truly novel phases is currently sparse, there is no doubt that they do exist, and the exploitation of oxide interfaces to create such novel phases holds promise as a route to new, exciting, and useful properties. Because this effect creates electronic phases wholly absent in the parent material regardless of electron concentration, we distinguish it from electronic reconstruction by calling it "electronic metamorphosis" [in (15, 17) the term "electronic reconstruction" has also been used to describe such effects].

A Route to New or Improved Technologies?

As a result of these and other phenomena, interfaces in oxides can harbor surprising electronic systems with remarkable properties. These systems are still topics of fundamental research. Yet for some of them the relevance to possible applications is already apparent, either because the interfaces provide potentially useful electronic properties or because the interfaces determine the electronic behavior of tech-

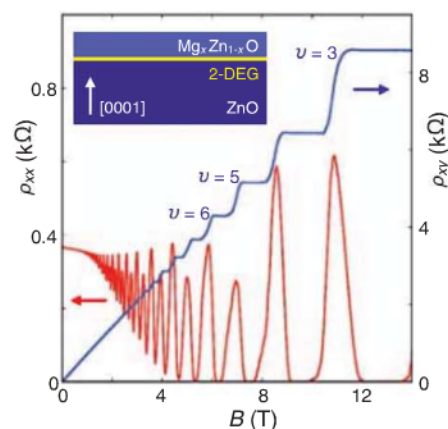


Fig. 2. Measured longitudinal resistance ρ_{xx} (red) and Hall resistance ρ_{xy} of a two-dimensional electron gas formed at a ZnO-Mg_{0.05}Zn_{0.95}O interface, where ν is the Landau filling index. The data were measured at 0.5 K. Data courtesy of A. Tsukazaki and M. Kawasaki [after (10)].

nologically relevant compounds. Grain boundary interfaces in the high-critical temperature (T_c) superconducting cuprates are one of the most important examples of the latter. These boundaries are charged, and the space charge-induced carrier density change triggers a phase transition of the superconductor into an insulating phase (19). As a result, nanometer-wide highly resistive layers are created at the boundaries—a textbook example of electronic reconstruction (Fig. 3B). Although these insulating layers are exploited to make high-quality Josephson junctions, they were a key problem for the fabrication of high- T_c superconducting cables, because the supercurrent flow is impaired by these regions, causing resistive losses in the superconductor. This technological roadblock was overcome in two ways: (i) by using grain shapes that provide large boundary areas and therefore wide current paths, as used for cables fabricated from Bi-based cuprates, or (ii) by aligning the grains to reduce the width of the insulating layer, as implemented in the second generation of high- T_c cables, the coated conductors fabricated from the 123-cuprates.

Electronic reconstruction has been used to generate interfacial superconductivity (20, 21) and also in the design and fabrication of ultrathin magnetic layers. Heterostructures of antiferromagnets were grown that electronically reconstruct to induce a ferromagnetic state precisely at the interface (22). Pushing ultrathin magnetic layers to the limit, strategies to generate two-dimensional electron systems that are fully spin-polarized have been conceived. These are based, for example, on LaAlO_3 - EuO interfaces (23).

Interfaces involving manganites have been intensively investigated to exploit the colossal magnetoresistance for which these materials are famous. Although manganites are already highly magnetoresistive in the bulk, the magnetoresistivity is enhanced by orders of magnitude at grain boundary interfaces (24). Space charge is once more important; it suppresses the local Curie temperature (25) and thereby enhances the magnetoresistance. These experiments highlight the extraordinary sensitivity with which interfacial electronic systems can react to external parameters—the basis of a sensor.

In contrast to the comparatively simple ferromagnetic interfaces in manganites used to enhance

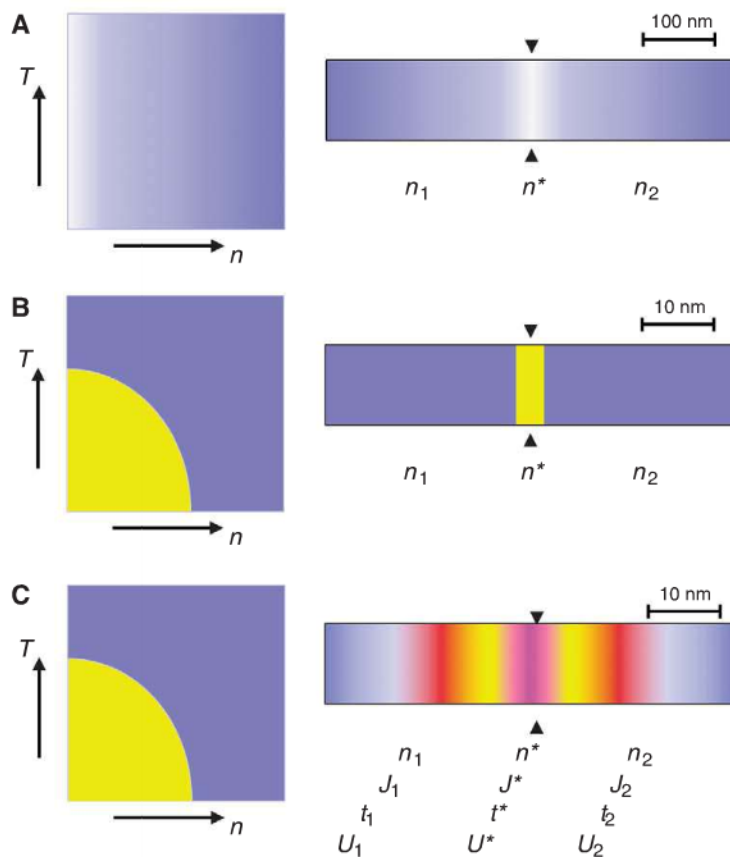


Fig. 3. Behavior of electronic systems at interfaces in solids. (A to C) Exemplary phase diagrams (left) and sketches of the electronic systems (right) for conventional semiconductors (A), correlated oxides with interfaces characterized by electronic reconstruction (B), and correlated oxides with interfaces generated by electronic metamorphosis yielding phases not found in the bulk (C). The interfaces are marked by arrowheads. For clarity, carrier freeze-out at low temperatures is not considered in the semiconductor phase diagram. In (A), the semiconductor interface is characterized by a space-charge layer (white) with depletion of carrier density n ; in (B), the charge depletion drives a phase transition to the phase of the bulk phase diagram drawn in yellow. In (C), the hopping energies t and correlation parameters (e.g., Coulomb repulsion energies U and exchange energies J) are altered from their bulk values inside the two materials (e.g., t_1 , t_2 , or U_1 , U_2) to a new set of values at the interface (e.g., t^* , U^*), so that novel electronic phases (colored stripes) are generated.

their magnetoresistivity, the interface between $\text{La}_{2/3}\text{Ca}_{1/3}\text{MnO}_3$ and the high- T_c superconductor $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ has truly surprising properties (26). Across this interface covalent bonds are formed, which modify the electronic structure of the CuO_2 layers of the $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$. As a result, the Cu ions in the $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ at the interface are ferromagnetically magnetized. Such order is impossible for bulk $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$. The possibility of using interfaces to alter materials is an intriguing means to design novel superconductors (27–29).

Conducting sheets at interfaces—which are generated between insulators, work at room temperature, can be tuned by electric fields applied by gate electrodes, and feature high carrier mobilities—represent an ideal configuration for electronic applications. The seminal discovery of conducting electron systems at interfaces between

the band insulators LaAlO_3 and SrTiO_3 comes close to realizing this (30). If LaAlO_3 is epitaxially grown on the TiO_2 -terminated and (100)-oriented surface of SrTiO_3 , a conducting electron-doped (n -type) sheet is generated at the interface. Remarkably, this happens only if the insulating LaAlO_3 is at least four unit cells thick (31). As a result, the conductivity can be precisely switched by adding or etching away single monolayers of the overlying LaAlO_3 insulator when the LaAlO_3 thickness in such heterostructures is just below or above the critical thickness for conductivity.

Typical sheet conductances of the electron system at the LaAlO_3 - SrTiO_3 interface are $5 \times 10^{-5} \text{ S}$ and $5 \times 10^{-3} \text{ S}$ at 300 K and 4.2 K, respectively. Mobilities are $\sim 7 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ at 300 K and $\sim 700 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ at 4.2 K. Carrier densities are usually in the range of $10^{13}/\text{cm}^2$. In the direction perpendicular to the interface, the conducting sheet is at most a few nanometers thick (32–36). These characteristics, which differ remarkably from the typical properties of semiconductor interfaces (table S1), are shown by samples that are grown at oxygen pressures of $5 \times 10^{-5} \text{ mbar}$ and are subsequently oxidized (e.g., during cooling) at close to 1 bar of oxygen. Under these conditions, the conducting sheet generated by electronic reconstruction is not shunted by electrons arising from doping of SrTiO_3 by oxygen vacancies (37). Yet even if such effects are avoided, the interfaces can be influenced by a variety of defects that depend on growth conditions, such as defect complexes involving oxygen vacancies, intermixing, and other off-stoichiometries that may even be inhomogeneous (38, 39).

Catastrophe and Reconstruction

Equivalent interfaces have also been found for several other material combinations, which, like LaVO_3 - SrTiO_3 multilayers (40), all involve SrTiO_3 as one component. A generic mechanism has been proposed to drive the generation of the conducting layers (41). This mechanism has acquired fame as the “polar catastrophe.” In growing, for example, a (100)-oriented LaAlO_3 film, an electrostatic voltage is built up as the charged LaO^+ and AlO^- planes of the LaAlO_3 are stacked in series. The voltage grows with the LaAlO_3 thickness to such large values that electrons reduce their Coulomb

energy by moving from the LaAlO_3 sheet to the interface, to occupy Ti 3d states there. These 3d states form two-dimensional bands extended parallel to the interface. As many degrees of freedom are involved, the generation of the conducting electron system is a complex process. It involves, for example, orbital reconstruction of the Ti 3d states (35) and subtle yet critical shifts of the ion positions (42, 43). Even if the possible defects listed above are not considered, the generation of the electron system can only be described with completeness numerically, such as by methods based on density functional theory (DFT) [see, e.g., (42–45)].

Scanning tunneling spectroscopy measurements of the density of states at the LaAlO_3 - SrTiO_3 interface (46) have revealed remarkable differences between this two-dimensional electron system and the 2DEGs formed at interfaces between conventional semiconductors. As sketched in Fig. 4A, at the semiconductor interface the mobile electrons move in two-dimensional subbands within the quantum well generated by band bending. At the oxide interface, however, there are multiple quantum wells given by the ionic potentials of the TiO_6 octahedra, and within each quantum well are subbands that are a subset of the Ti 3d states (Fig. 4B). In these bands, the electrons are subject to the correlations of the Ti 3d orbitals and form a two-dimensional electron liquid rather than an electron gas (46). Being a metal at room temperature, the electron liquid condenses below ~ 250 mK into a two-dimensional superconducting state (20).

At all temperatures, the carrier density in these sheets reacts sensitively to gate fields with concomitant changes of the conductivity. Indeed, large, depleting gate fields switch the systems into a completely insulating phase. Even the superconducting ground state can be switched into an insulating one, crossing a quantum critical point at several multiples of $10^{13}/\text{cm}^2$. As small variations of gate voltage cause drastic resistance changes, electric field-driven phase transitions are of interest for use in drain source channels of field-effect transistors to enhance the channels' transport response to gate fields.

The electron liquid at the LaAlO_3 - SrTiO_3 interfaces is robust and stable up to several hundred degrees Celsius. This enables samples to be contacted and patterned with standard lithography techniques (8) (Fig. 1D). This electron system can also be patterned on the nanometer scale, well beyond the limits of standard lithography, using an atomic force microscope (AFM) (9). By biasing an AFM tip with a positive voltage of 10 V, conducting lines have been written in 3-monolayer-thick LaAlO_3 - SrTiO_3 (by default an insulating electron system) with a spatial resolution as fine as 2 nm. With the use of negative voltages, the lines can be erased again. Lifetimes of days have been achieved, and nanometer-sized exploratory devices have been written (9).

Quantum confinement has also been predicted for vanadate-based interfaces, and this phenomenon is expected to generate electronic properties even more peculiar than those of LaAlO_3 - SrTiO_3

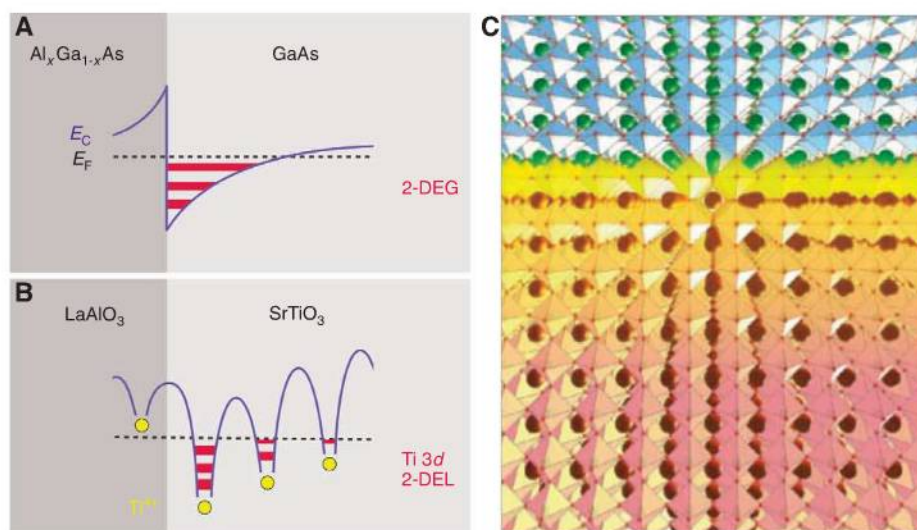


Fig. 4. (A and B) Comparison between two-dimensional electron systems generated at interfaces between standard semiconductors, for example, between GaAs and $\text{Al}_x\text{Ga}_{1-x}\text{As}$ (A) and between LaAlO_3 and SrTiO_3 (B). At the semiconductor interface, two-dimensional electronic subbands are formed within the quantum well generated by band bending. At the oxide interface, the two-dimensional bands are generated in the potential wells arising predominantly from the ionic charges. For the LaAlO_3 - SrTiO_3 interface, these are 3d bands of Ti ions. The overall band bending lowers the electronic energies of the unit cells next to the interface, such that the mobile electron system that forms a two-dimensional electron liquid (2DEL) resides predominantly in the oxide planes next to the interface [after (46)]. (C) Illustration of the situation in (B): The LaAlO_3 is grown on top of the SrTiO_3 ; the mobile electron system is depicted in yellow.

interfaces: Quantum confinement in half-metallic VO_2 layers embedded within insulating TiO_2 is predicted to produce an unprecedented two-dimensional state characterized by a (semi-Dirac) point Fermi surface. This state comprises spinless charge carriers that have a finite effective mass along one principal axis of the lattice but are massless along the other (47).

Oxide interfaces are also of interest for their possible multiferroic behavior and for their thermoelectric properties. Large thermoelectric voltages have been reported for two-dimensional electron systems in $(\text{Nb,Sr})\text{TiO}_3$ - SrTiO_3 quantum wells (48). The stated Seebeck voltages are very high ($850 \mu\text{V/K}$). For applications, such large voltages are highly desirable. In principle, the use of superlattices enables many interfaces to be added in parallel to achieve sizable power output. Novel device architectures are desired, in order to decrease the thermal conduction of the materials forming the interfaces.

Outlook, Challenges, and Potential Pitfalls Ahead

The spectacular phenomena already discovered at oxide interfaces—self-generated ultrathin magnetic layers, orbitally reordered electronic systems, conducting electron gases that can be patterned into nano-sized transistors—are of tremendous fundamental interest. Yet these phenomena also bear great potential for applications. The exploration of interfacial electronic systems is, however, in its infancy. Only in a few cases, such as high- T_c superconducting cables and Josephson junctions, are concrete applications in existence or close at hand. The exploration of interfaces in complex oxides is a

nascent field of research in electronics—a field that has vast potential for applications. Many scientific questions remain to be resolved and discoveries to be made. In the present state of the field, the basic science must be understood before the focus can shift toward devices.

Consider the LaAlO_3 - SrTiO_3 interfaces, for example. Although the feature sizes and on-off ratios are magnificent, the room-temperature mobilities are orders of magnitude below any desirable value. Interestingly, it is not even clear whether the mobilities are limited by sample quality or whether they, as one might expect, can be enhanced by the use of different material combinations or by altering the sample structure. Likewise, switching speeds and noise figures are unknown. The fact that in many cases LaAlO_3 - SrTiO_3 bilayers grown under varying conditions by several groups show similar properties reveals that the basic properties of these interfaces are robust, yet it will never be possible to grow the ideal LaAlO_3 - SrTiO_3 interface. Defects such as cation nonstoichiometries, oxygen defects, intermixing, and nonhomogeneities will be present in the samples, but to different degrees [see, e.g., (37–39)]. Abundant pitfalls will result if defects discovered in one sample are associated with electronic or structural properties measured in another sample that was grown differently. An improved control and understanding of defects will also be necessary for another big advance: the fabrication of superlattice stacks containing several conducting interfaces, ideally also including p -type ones (49), that can be locally interconnected as illustrated in fig. S1. Such structures, which may also comprise other functional

oxide materials, such as ferroelectrics or strongly correlated materials, may not only have intriguing electronic properties but will also greatly enhance our experimental capabilities in investigating the interface properties, for example, by diffraction techniques. The samples contain many tunable interfaces in parallel, and therefore provide enhanced diffraction intensities. These structures will also enable new transport studies—for example, true four-point measurements perpendicular to the interfaces or measurements of the coupling between interface electron systems.

It is furthermore desirable to generate conducting interfacial electron systems not only on single crystalline SrTiO₃ substrates but also, for example, on large silicon wafers coated with SrTiO₃, or—even better—coated with an oxide that yields interfaces that have properties superior to those of LaAlO₃-SrTiO₃, that can be fabricated cost-efficiently, and that are compatible with semiconductor fabrication processes.

Fortunately, the vast materials palette of oxides provides virtually unlimited possibilities to design and fabricate interfaces with functional properties. Also, just as individual functional interfaces can be generated by electronic metamorphosis, it should also be possible to create new artificial bulk materials. Such materials could be fabricated using superlattices or electronically active interfaces at nanoscale inhomogeneities. Because the electronic systems produced are intrinsically inhomogeneous in the direction perpendicular to the interfaces, new freedom is provided to design novel electronic materials. One proposal, for example, involves the fabrication of superconductors with greatly enhanced critical temperatures by spatially decoupling the mobile charge carriers (flowing, e.g., at the interface plane) from the pairing interaction (provided, e.g., by polarizations of an adjacent compound) [see, e.g., (27, 28)] or by using the interface to tailor the orbital structure of the superconductor-to-be (29). Interfaces and interface-based functional materials are also relevant for switching and sensor applications because the interfacial electron systems in the oxides characteristically have competing ground states of similar energies, among which the systems can be switched by currents, external fields, or adsorbed species.

The theoretical modeling of interfaces in correlated systems is another area ripe with opportunity. The ability to construct oxide interfaces with atomic-scale precision has made huge strides in recent years, but what interfaces should be grown to realize new phenomena, greatly enhanced properties, and useful devices exploiting these effects? This is a gold mine begging for materials-by-design solutions using the full arsenal of simulation, modeling, and theory. The polar catastrophe model presents a main driving force for electronic reconstruction or metamorphosis, but it is not a comprehensive microscopic model, for it presents only one aspect of the interface physics. Tremendous progress has been made in the development of DFT-

based models, and further advances are needed to enhance the size of their supercells so that microstructural defects can also be considered. We expect such models to provide explanations for the differences between experimental data and predictions based on the bare polar catastrophe model, such as the order-of-magnitude difference between the measured and catastrophe-predicted density of mobile carriers at the LaAlO₃-SrTiO₃ interface. Finally, interface models based on dynamical mean field theory need to be advanced to correctly take the important correlation effects into account [see, e.g., (17, 50)].

Concluding Remarks

Silicon metal oxide semiconductor field-effect transistors (MOSFETs) are the workhorses of mainstream electronics and will continue in this role for the foreseeable future. One means to boost their performance and to overcome the quantum mechanical limitations of miniaturization is to accent the capabilities of silicon with functional oxides. The replacement of SiO₂ with a highly polarizable Hf-based oxide gate insulator (51) was the first important step, one that heralds a more generic development. With increasing miniaturization, the characteristic energies of electronic circuits are reaching the quantum mechanical regime. Switching energies of standard devices such as MOSFETs are becoming comparable to the correlation energies of the electron systems in the materials of the devices as described in (52). New devices are needed, such as dielectric capacitors with capacitances exceeding textbook limits (52). The state of this research is reminiscent of semiconductor physics half a century ago. As was the case for semiconductor technology back in the 1950s, the challenge to understand, predict, and tailor the physical properties of interfaces between complex oxides is enormous. Incorporation of functional oxides into electronic circuits or their use in other applications requires mastering these interfaces. Today we are in the midst of learning how to meet this challenge; once mastered, these interfaces will provide vast and unforeseen opportunities, technologies, and science for decades to come.

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Fig. S1

Table S1

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A Southern Tyrant Reptile

Roger B. J. Benson,^{1*} Paul M. Barrett,² Tom H. Rich,^{3,4} Pat Vickers-Rich^{3,4}

The 100-million-year tyrannosauroid lineage is poorly documented. Its youngest representatives, the deep-skulled, multiton tyrannosaurids, were the apex predators of latest Cretaceous Laurasia. These are known from abundant, well-preserved fossils. Until recently, however, almost nothing was known about the ecology and biogeography of earlier tyrannosauroids. This situation is changing. A surge of new discoveries is revealing diverse ecotypes and body sizes from as early as the Middle to Late Jurassic [e.g., (1–3)]. Despite this, the record of tyrannosauroid evolutionary history has been limited to the northern

continents (Fig. 1A), an aberrant pattern given the broad distributions of other long-lived dinosaur clades. Here we report an Australian tyrannosauroid, represented by a pubis from the late Early Cretaceous of Victoria (Fig. 1, B and C) (4, 5) [National Museum of Victoria (NMV) P186046].

The pubis is almost identical to those of tyrannosaurids (Fig. 1, B to D). Several distinctive synapomorphies indicate tyrannosauroid affinities. The transversely narrow, parallel-sided pubic boot indicates referral to Coelurosauria (6). The pubic tubercle is broken, but the preserved portion indicates a prominent, anterolaterally curving, flange-

like morphology, as in tyrannosaurids (Fig. 1D) and dromaeosaurids (7) (many other features distinguish the dromaeosaurid pubis). This is confirmed by the presence of a rugose lateral surface adjacent to the tubercle, also present in tyrannosaurids. The pubic boot is large; its anteroposterior length is 0.45 times the pubic shaft length (a minimum estimate because the boot is broken). This is comparable to those of tyrannosauroids and some basal coelurosaurs, although adult tyrannosaurids possess a still larger pubic boot (Fig. 1D; ratio = 0.65). The anterior expansion of the boot is substantial, as in tyrannosaurids but unlike basal tyrannosauroids and most other coelurosaurs (fig. S1). Although allosauroids have a large pubic boot, it is transversely broad, characteristic of non-coelurosaurian theropods (6) (fig. S1), and the pubic tubercle is moundlike. An anterior expansion is also present in some ornithomimosaurs and oviraptorosaurs, but these have a small pubic boot and low pubic tubercle (fig. S1), unlike NMV P186046.

These observations show that NMV P186046 is similar to tyrannosauroids in general and shares derived features with tyrannosaurids specifically (Fig. 1A). It is derived compared with other Early Cretaceous tyrannosauroids, including *Raptorex*, in which the pubic tubercle is not flange-like or rugose. Thus, advanced cranial and appendicular characters linking *Raptorex* and tyrannosaurids (2) may be inferred for NMV P186046. This demonstrates that advanced tyrannosauroids with characteristic short arms and powerful jaws achieved a global distribution in the Early Cretaceous. The length of NMV P186046 (307 mm) is only slightly longer than the pubis of *Raptorex* [279 mm (2)]. Thus, a potentially cosmopolitan grade of small tyrannosauroids with a tyrannosaurid-like body plan preceded the Late Cretaceous rise of the colossal tyrannosaurids.

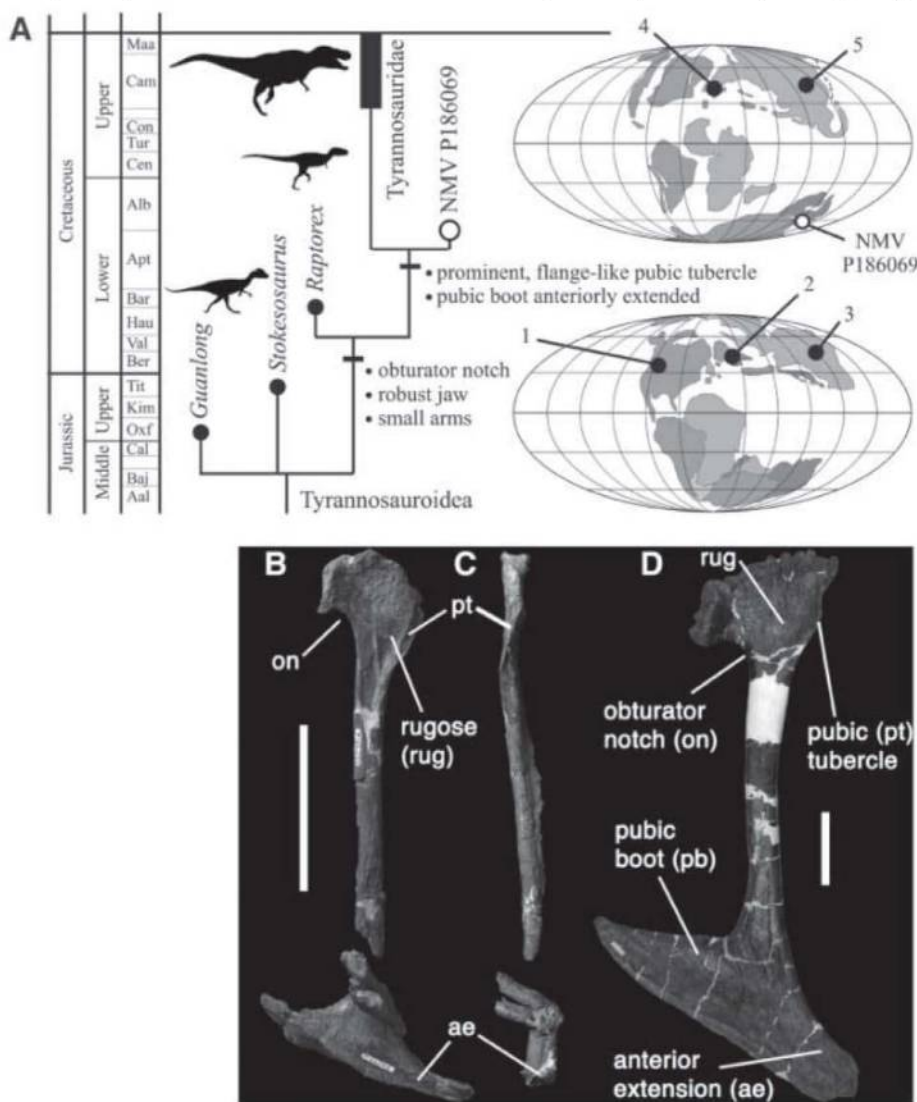


Fig. 1. (A) (Right) Distribution of Late Jurassic (bottom) and Early Cretaceous (top) tyrannosauroids: 1 and 2, *Stokesosaurus*; 3, *Aviatyrannis*; 4, *Guanlong*; 5, *Dilong*, *Raptorex*, *Sinotyrannus*, and *Xiongguanlong*. (Left) Relationships of NMV P186046 to other tyrannosauroids for which the pubis is known, based on (2). (B to D) Tyrannosauroid pubes: NMV P186046 in right lateral (B) and anterior (C) views; albertosaurine tyrannosaurid (Royal Tyrell Museum of Palaeontology, Drumheller no. 1986.64.1) in right lateral view (D). Scale bars equal 100 mm.

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Fig. S1

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Doc2b Is a High-Affinity Ca^{2+} Sensor for Spontaneous Neurotransmitter Release

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Synaptic vesicle fusion in brain synapses occurs in phases that are either tightly coupled to action potentials (synchronous), immediately following action potentials (asynchronous), or as stochastic events in the absence of action potentials (spontaneous). Synaptotagmin-1, -2, and -9 are vesicle-associated Ca^{2+} sensors for synchronous release. Here we found that double C2 domain (Doc2) proteins act as Ca^{2+} sensors to trigger spontaneous release. Although Doc2 proteins are cytosolic, they function analogously to synaptotagmin-1 but with a higher Ca^{2+} sensitivity. Doc2 proteins bound to *N*-ethylmaleimide-sensitive factor attachment receptor (SNARE) complexes in competition with synaptotagmin-1. Thus, different classes of multiple C2 domain-containing molecules trigger synchronous versus spontaneous fusion, which suggests a general mechanism for synaptic vesicle fusion triggered by the combined actions of SNAREs and multiple C2 domain-containing proteins.

Neurotransmitter release is triggered by a rise in intracellular Ca^{2+} , which activates sensors that subsequently trigger vesicle fusion. Synchronous release, the fastest mode of neurotransmission, involves the Ca^{2+} sensors synaptotagmin-1, -2, or -9, which are anchored in the vesicle membrane and contain two cytoplasmic C2 domains that bind phospholipids in a

Ca^{2+} -dependent manner and interact with the soluble *N*-ethylmaleimide-sensitive factor attachment receptor (SNARE) complex (1–5). Synaptotagmin-1-deficient neurons lack synchronous release but display an increase in spontaneous release (6–9) except in autapses (1, 10), which suggests a distinct mechanism for spontaneous release. Spontaneous release occurs in the absence of action

potentials and is largely Ca^{2+} dependent (11–15), although truly Ca^{2+} -independent fusion may also exist (16).

Doc2a and Doc2b are soluble proteins that contain C2 domains, which are similar to synaptotagmins (17, 18). They are expressed in nerve terminals and interact with the secretory molecules Munc18 and Munc13 and the SNARE proteins syntaxin-1 and SNAP25 (the synaptosome-associated protein of 25 kD) (19, 20). Overexpression of Doc2b enhances exocytosis in chromaffin cells

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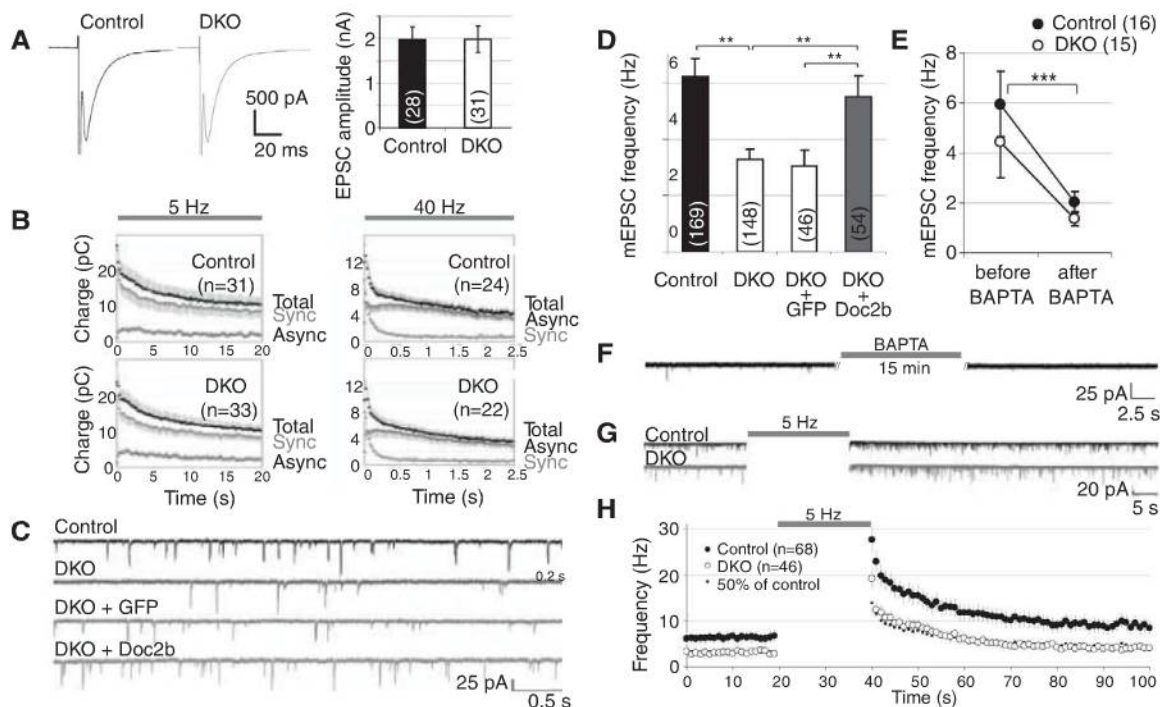
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Fig. 1. Impaired spontaneous neurotransmission in Doc2a- and b-deficient mice. Hippocampal neurons were cultured on microislands to promote self-innervation. (A) Averaged evoked EPSCs in control and DKO neurons. (B) Evoked EPSC charge (mean $\pm$ SEM) during repeated stimulation at 5 or 40 Hz. The synchronous and asynchronous components were estimated as previously reported (25). (C) Representative traces of spontaneous EPSCs in wild-type or DKO cells. To rescue the phenotype, GFP or Doc2b was acutely expressed in DKO cells. (D) Average spontaneous release frequency in control and DKO cells and rescue by Doc2b overexpression. Cell numbers are indicated between brackets. ** $P < 0.01$. (E) Average mEPSC frequency before and after intracellular loading of the Ca^{2+} chelator BAPTA. The average spontaneous release rate varied between experiments, but our experimental design prevents confounding effects thereof (fig. S2E). (F) Example trace representative for the data in (E), taken from a DKO cell before and



after BAPTA loading. (G) Typical recordings before and after repetitive stimulation at 5 Hz. (H) Individual release events were binned in 1-s intervals to monitor their average frequency immediately after repetitive stimulation. Squares were calculated as 50% of the frequency in control cells.

(19), pancreatic beta cells (21, 22), and adipocytes (23), but its function in neurons is elusive.

Role of Doc2b and Ca^{2+} in spontaneous synaptic release. We generated *Doc2b*^{-/-} mice by deleting the promoter and exon 1 of the *Doc2b* gene (fig. S1) (24). *Doc2b*^{-/-} mice did not express the remaining exons and lacked Doc2b immunoreactivity. *Doc2b*^{-/-} mice were viable and fertile

without gross abnormalities. Other proteins implicated in neurotransmitter secretion were expressed at normal levels (fig. S1D). Compensatory ectopic expression of *Doc2a* was not detected in Doc2b-deficient brains by in situ hybridization and the Doc2a protein level was unchanged (fig. S1). *Doc2a*^{-/-} *Doc2b*^{-/-} double knock-out (DKO) mice were also viable, fertile, and indistinguishable with

regard to gross anatomy. To study neurotransmission and synaptic plasticity in Doc2 mutants, hippocampal neurons were cultured on glial microislands to promote self-innervation (autapses). Because Doc2a and Doc2b are both expressed in the hippocampus (18), DKO mice were compared with wild-type controls. All aspects of evoked release were normal, with excitatory postsynaptic currents (EPSCs) of normal amplitude and shape (Fig. 1A), a normal synaptic depression during prolonged stimulation at 5 or 40 Hz (Fig. 1B and fig. S2, A and B), a normal contribution to the total postsynaptic charge transfer (25) of asynchronous release (Fig. 1B) and a normal size of the readily releasable pool (RRP) (fig. S2D).

However, DKO mice exhibited a reduction in the spontaneous release frequency to half that of controls (6.2 Hz in wild type to 3.3 Hz in DKO) (Fig. 1, C and D). Acute expression of Doc2b in DKO neurons restored this phenotype. Doc2b immunoreactivity was confirmed in all cellular compartments, including synapses, at the time of analysis (fig. S3). As a control, acute expression of green fluorescent protein (GFP) in DKO cells did not affect the spontaneous release frequency (3.1 Hz) (table S1). A normal organization of the postsynaptic apparatus was indicated by a normal miniature EPSC (mEPSC) amplitude, as well as normal rise time and decay time in DKO neurons (fig. S2F) or after overexpression (fig. S3B).

Because Doc2 proteins require Ca^{2+} for their phospholipid-binding and secretion-enhancing activity (19, 26), we investigated whether spontaneous release also depended on Ca^{2+} . The spontaneous release frequency was measured in the same cells before and after loading them with the Ca^{2+} che-

Fig. 2. Reduced frequency of spontaneous events in Purkinje cells lacking Doc2b. (A) Doc2b mRNA was detected by in situ hybridization in cerebellar Purkinje cells from wild-type, but not *Doc2b*^{-/-} (KO) mice. (B) Typical voltage-clamp recordings in acute slices. (C) Mean frequency of spontaneous miniature inhibitory postsynaptic currents (mIPSCs) in KO mice and age-matched control littermates ($n = 15$ and 28 cells from two and three mice, respectively). $***P < 0.0001$. (D and E) Representative current clamp recordings of Purkinje cell firing patterns in controls (D) (eight cells from three mice) and KO cells (E) (seven cells from two mice).

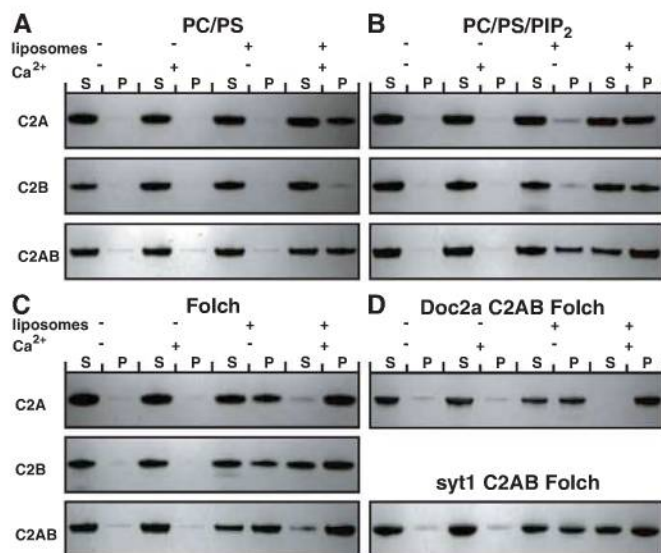
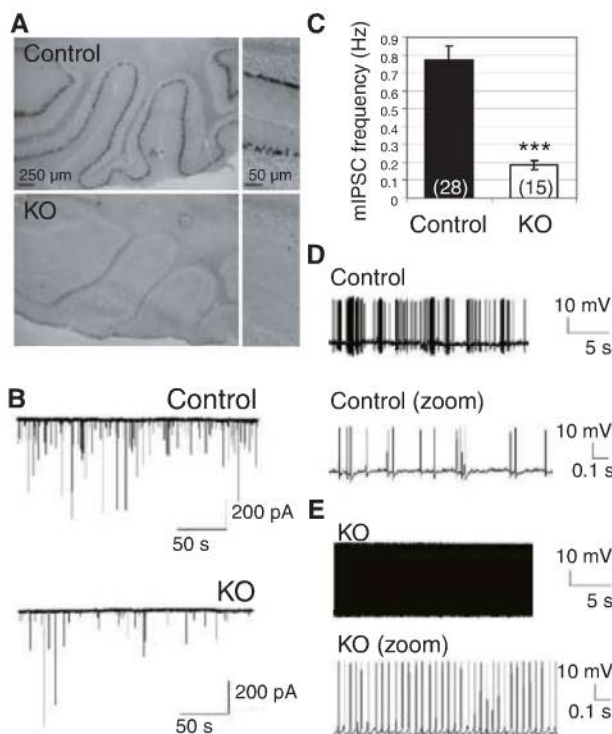
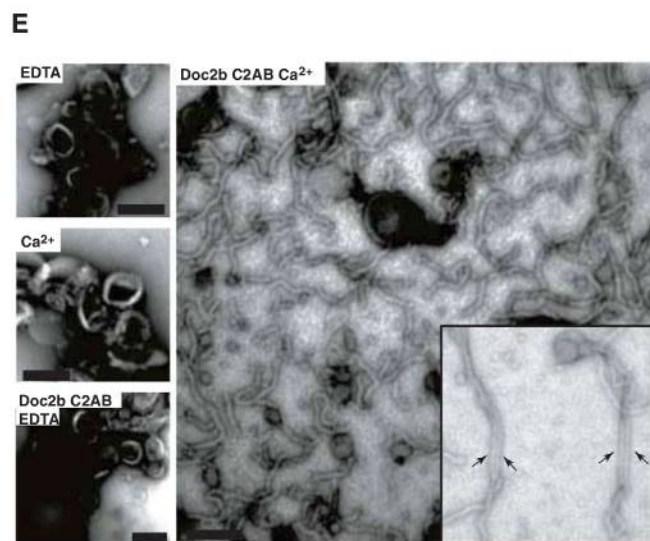


Fig. 3. Membrane-binding and curvature induction by Doc2b. (A to D) Liposome cosedimentation assays to characterize the membrane-binding properties of the Doc2b C2A, C2B, C2AB, Doc2a C2AB, and synaptotagmin-1 (Syt1) C2AB domains. Liposomes composed of the indicated lipids were incubated with the Doc2b C2A, C2B, or C2AB domains. Liposome-bound protein was cosedimented by ultracentrifugation. Eight percent of the supernatant and of the pellet fraction was run on 4 to 12% gradient gels. Proteins were visualized by Coomassie staining. S indicates unbound protein in the supernatant; P



indicates liposome-bound, and thus copelleted, protein. No or very little protein sedimented in the absence of liposomes. (E) Ca^{2+} -dependent tubulation of liposomes by the Doc2b C2AB domain. Folch liposomes were incubated in the absence or presence of Doc2b C2AB and/or Ca^{2+} and processed for electron microscopy by negative stain. The arrows indicate bundles of closely aligned tubules. Scale bar, 100 nm. All data shown are representative of at least three independent experiments. PS, phosphatidylserine; PC, phosphatidylcholine.

lators 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA). This significantly reduced the frequency to 2.0 Hz ($P < 0.0001$) in wild-type cells and to 1.4 Hz in DKO cells ($P < 0.0001$) (Fig. 1, E and F). The frequency did not decrease further after longer incubation with BAPTA-AM (a membrane-permeable form of BAPTA) (fig. S2H). Thus, as expected (12–15), spontaneous events are often triggered by short-lived Ca^{2+} transients in resting cells.

Consistent with the role of Ca^{2+} in spontaneous release, the frequency of individual release events markedly increased after intense activity (5 Hz for 20 s) (Fig. 1, G and H) and decayed after the last stimulus with an exponential $t_{1/2}$ of 11 s. Similar observations have been made in the neuromuscular junction and calyx of Held (27, 28). In DKO cells after intense stimulation, the number of observed events was reduced to 50% of wild type, except for the first time point that was

sampled 200 to 1200 ms after the last depolarizing stimulus, where asynchronous release may still contribute (Fig. 1H). The Doc2-independent events remaining in DKO cells were also affected by BAPTA and repetitive firing, which suggested that additional Ca^{2+} -dependent mechanisms exist besides Doc2a- and b-dependent events. Thus Doc2 proteins responding to intracellular Ca^{2+} are required for approximately half of the spontaneous release events in hippocampal neurons.

Disinhibition in Purkinje cells lacking Doc2b.

Doc2b mRNA was abundant in Purkinje cells (PCs) of the cerebellum (Fig. 2A), whereas *Doc2a* was not detectable. *Doc2b* expression was exclusive to the PC layer with no detectable mRNA in other cerebellar cells including interneurons. PCs synapse onto neighboring PCs via recurrent axon collaterals (29). We performed whole-cell voltage-clamp recordings at postnatal days 7 to 8 because at this time, recurrent synapses are the predomi-

nant source of GABA-mediated (GABAergic) input, whereas stellate and basket cells are still functionally immature (30). In the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX) to block AMPA receptors and tetrodotoxin (TTX) to block sodium currents, the PCs had a stable resting potential without *N*-methyl-D-aspartate receptor currents or Ca^{2+} spikes. Under these conditions, any remaining inhibitory postsynaptic currents (IPSCs) can be interpreted as spontaneous release events.

The frequency of spontaneous IPSCs was reduced to one-fourth in *Doc2b*^{−/−} mice compared with control littermates (Fig. 2, B and C). Postsynaptic parameters were normal (i.e., amplitude, rise time, and decay). In young rodents, GABAergic input inhibits PC firing (30), and recurrent PC-PC synapses are the major GABA source (31). We thus tested if the disinhibition in *Doc2b*^{−/−} PC-PC synapses affected PC spiking. Whole-cell current clamp recordings were per-

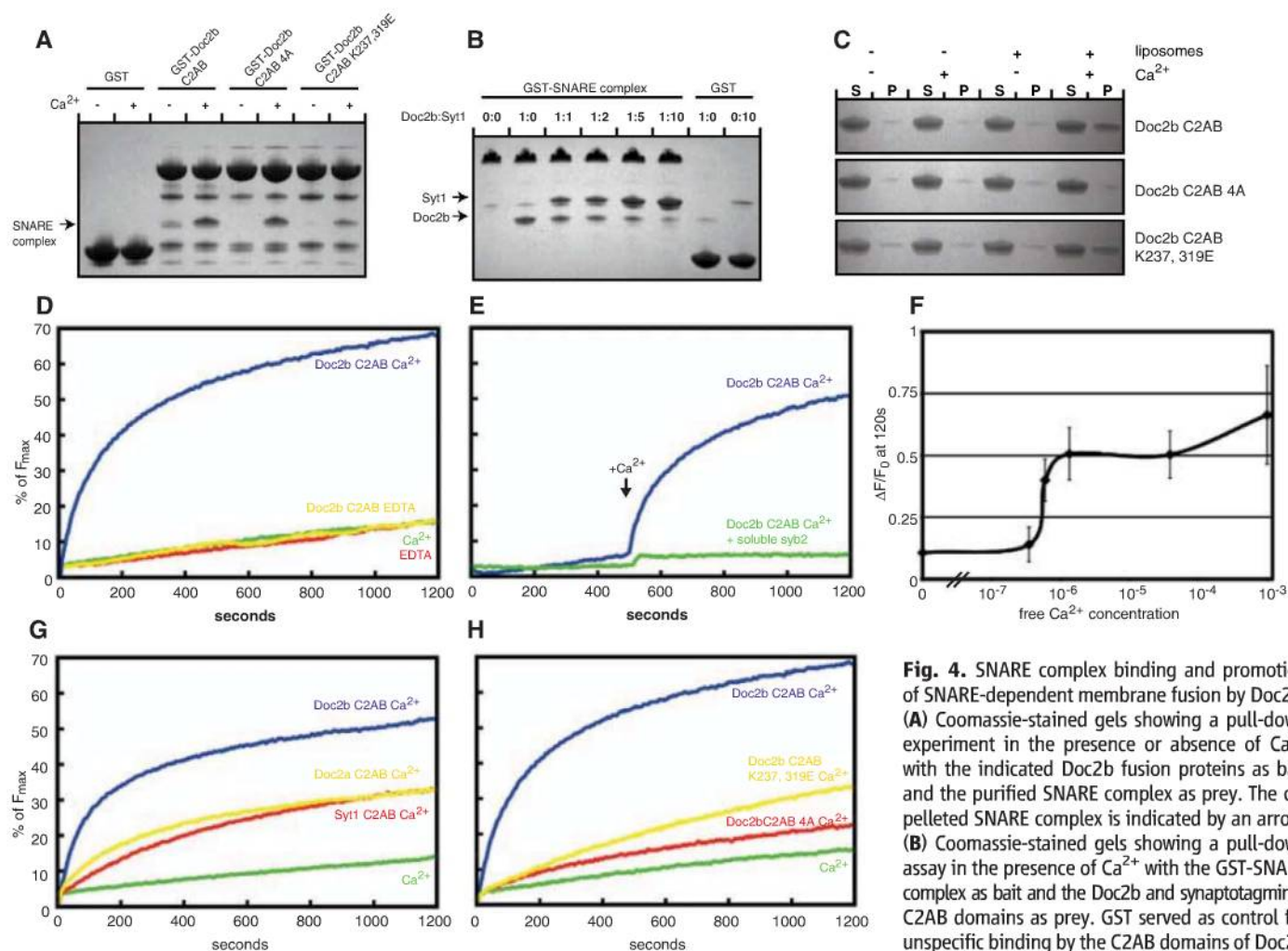


Fig. 4. SNARE complex binding and promotion of SNARE-dependent membrane fusion by Doc2b. (A) Coomassie-stained gels showing a pull-down experiment in the presence or absence of Ca^{2+} with the indicated Doc2b fusion proteins as bait and the purified SNARE complex as prey. The copelleted SNARE complex is indicated by an arrow. (B) Coomassie-stained gels showing a pull-down assay in the presence of Ca^{2+} with the GST-SNARE complex as bait and the Doc2b and synaptotagmin-1 C2AB domains as prey. GST served as control for unspecific binding by the C2AB domains of Doc2B and synaptotagmin-1, respectively. The copelleted

Doc2B and synaptotagmin-1 C2AB domains are indicated by arrows. (C) Liposome cosedimentation assay in the presence or absence of Ca^{2+} with the indicated proteins and liposomes composed of 20% PS, 70% PC, and 10% cholesterol. (D) In vitro membrane fusion assay with reconstituted full-length SNAREs in the absence or presence of Ca^{2+} and/or 7.5 μM Doc2b C2AB. (E) In vitro membrane fusion assay as in (D) but in the presence or absence of the soluble SNARE domain of synaptobrevin. Ca^{2+} was added at 500 s. (F) Ca^{2+} dose-dependence curve showing the change of fluorescence at 120 s in the reconstituted fusion assay in the presence of 7.5 μM Doc2b. Doc2b efficiently promotes fusion at submicromolar Ca^{2+} concentrations. The graph summarizes data from three independent experiments. (G) Comparison of the fusion-promoting activities of the Doc2b, Doc2a, and synaptotagmin-1 C2AB domains. (H) Fusion experiment using the indicated Doc2b C2AB proteins reveals that Ca^{2+} -dependent membrane and SNARE-binding are required for efficient fusion promotion. All data shown are representative of at least three independent experiments.

formed at postnatal day P17 in the absence of DNQX or TTX. We observed various firing patterns in PCs from wild-type or heterozygous mice: irregular trains of simple spikes in most cells (Fig. 2D) or trimodal firing patterns composed of tonic, burst, and silent periods, as expected (31, 32). In contrast, PCs from *Doc2b*^{-/-} mice showed continuous spiking without interruptions (Fig. 2E). This pattern occurred with a frequency of 20 to 25 Hz and was observed in all *Doc2b*^{-/-} cells tested, but never in control cells.

We also investigated neurotransmission in the calyx of Held synapse, a giant glutamatergic synapse in the auditory brainstem where the Doc2-Munc13 interaction is suggested to contribute to presynaptic plasticity (33). DKO mice exhibited normal evoked responses with a lower spontaneous release frequency by a factor of two (1.9 Hz compared with 4.8 Hz in control littermates), but this difference did not reach statistical significance (fig. S4).

Phospholipid and SNARE interactions. Doc2b is structurally similar to synaptotagmin-1 but lacks a transmembrane domain, and its C2 domains have a relatively high Ca²⁺ affinity (26). It is thus conceivable that Doc2b mediates spontaneous release events similar to synaptotagmin-dependent synchronous release, only with slower kinetics and a higher Ca²⁺ sensitivity. We thus tested if Doc2b is biochemically similar to synaptotagmin-1.

The Doc2b C2A domain bound in a Ca²⁺-dependent manner to phosphatidylserine-containing membranes (Fig. 3A) (34, 35). In addition, the C2B domain showed weak Ca²⁺-dependent phospholipid binding (Fig. 3A). The C2AB fragment of Doc2b showed stronger liposome binding than the isolated C2A and C2B domains (Fig. 3A). Ca²⁺-dependent membrane binding by synaptotagmin-1 was enhanced in the presence of phosphatidylinositol 4,5-bisphosphate (PIP₂) (36). For the C2A and C2B domains of Doc2b, the inclusion of PIP₂ enhanced both Ca²⁺-dependent and Ca²⁺-independent binding (Fig. 3B). This Ca²⁺-independent binding was localized to two polybasic patches on the C2A and C2B domains, respectively (figs. S5 and S6). The use of brain-derived Folch lipids (37) generally increased binding, including the Ca²⁺-independent

binding by the C2A domain (Fig. 3C and fig. S6). The C2AB domains of Doc2a and synaptotagmin-1 displayed similar behavior (Fig. 3D).

The C2AB domains of synaptotagmin-1, -3, and -5 induce a high degree of positive membrane curvature in a Ca²⁺-dependent manner (3), owing to the insertion of the tips of both C2 domains into the hydrophobic phase of the membrane. Shallow insertions into a monolayer are known to induce bending of the membrane toward the insertion (38–40). In order to examine if Doc2b also induces membrane curvature, we incubated liposomes derived from Folch lipids with 10 μM Doc2b C2AB domain in the absence (EDTA only) or presence of Ca²⁺ (Fig. 3E). Indeed, the Doc2b C2AB domain efficiently induced tubulation of otherwise spherical liposomes in a strictly Ca²⁺-dependent manner (Fig. 3E). We frequently observed closely aligned tubules, which indicated that the Doc2b C2AB domain could cluster liposomes in addition to their tubulation. Tubulation was still apparent at a Doc2b concentration of 5 μM but was barely detectable below 1 μM.

Synaptotagmin-1 requires SNARE complex binding to promote fusion in vitro and in vivo (4, 5). We thus tested if Doc2b also bound SNARE complexes. In one set of experiments we assembled the SNARE core complex on glutathione beads with the synaptobrevin SNARE domain fused to glutathione *S*-transferase (GST) (fig. S7). The Doc2b and synaptotagmin-1 C2AB domains were subsequently added in the presence or absence of Ca²⁺. In another set of experiments we used the GST-Doc2b C2AB domain to pull down the soluble SNARE complex (Fig. 4A). As expected (41), we found efficient Ca²⁺-dependent binding of the synaptotagmin-1 C2AB domain to SNARE complexes (fig. S7). The Doc2b C2AB domain also bound to SNARE complexes (Fig. 4A and fig. S7). In the C2B domain of synaptotagmin-1, the same poly-lysine motif that binds PIP₂ mediates SNARE complex binding (36). We thus tested SNARE complex binding by the mutant C2AB^{K237,319E} domain of Doc2b (42). As expected, it showed a profound loss of SNARE complex binding (Fig. 4A and fig. S7). Next, we tested if the putative membrane inserting hydro-

phobic amino acids in the tips of the Ca²⁺ binding loops (Fig. 4A and fig. S7) contributed to SNARE complex binding. To this end we mutated H158, F222, and I360A. The first Ca²⁺ binding loop of the Doc2b C2B domain contains an alanine and so was not mutated. We refer to this mutant as the 4A mutant. It showed no defect in SNARE complex binding, which indicated that, as for synaptotagmin-1, SNARE complex and membrane binding can occur at the same time (Fig. 4A and fig. S7). In contrast, Ca²⁺-dependent liposome binding was affected by only the 4A but not the K237,319E mutation (Fig. 4C). The decreased binding of the 4A mutant was less pronounced if the liposomes were made with higher percentages of phosphatidylserine.

The high structural and biochemical similarities between Doc2b and synaptotagmin-1 suggest that the binding site on the SNARE complex may be identical. We therefore tested if the C2AB domain of synaptotagmin-1 competes with the C2AB domain of Doc2b for SNARE complex binding (Fig. 4B). Indeed, increasing amounts of the synaptotagmin-1 C2AB domain competed away the Doc2b C2AB domain, which suggested that the binding sites for synaptotagmin-1 and Doc2b are overlapping.

Ca²⁺-dependent promotion of membrane fusion by Doc2b. The C2AB domains of several members of the synaptotagmin family promote fusion of liposomes containing reconstituted SNAREs in a Ca²⁺-dependent manner (43). Similarly, the C2AB domain of Doc2b promoted fusion of SNARE-containing liposomes, and this activity was strictly Ca²⁺ dependent (Fig. 4, D to H). When Ca²⁺ was introduced at a later time, the fusion-promoting effect was even more apparent (Fig. 4E). The fusion-promoting activity of Doc2b was SNARE dependent (Fig. 4E). If Doc2b acts as a Ca²⁺ sensor for spontaneous synaptic vesicle fusion, it is likely to be activated by intracellular Ca²⁺ in the submicromolar range (26). Consistent with this, Doc2b bound to membranes with a submicromolar affinity (fig. S8), and at submicromolar Ca²⁺, Doc2b promoted fusion (Fig. 4F). The in vitro fusion-promoting effect of Doc2b far exceeded that of synaptotagmin-1 and Doc2a (Fig. 4G). Next, we tested if Ca²⁺-dependent mem-

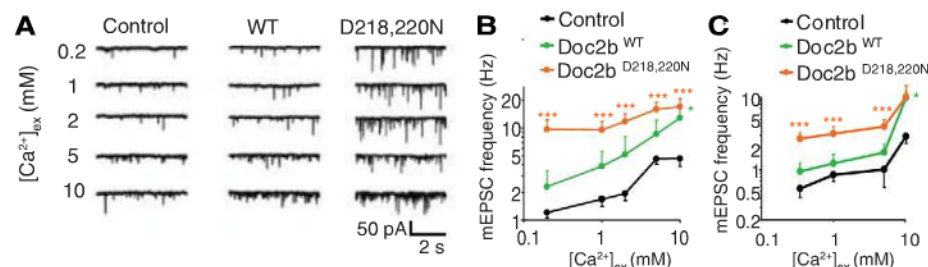


Fig. 5. The Ca²⁺ dependence of Doc2b determines that of spontaneous release in rescued DKO neurons. (A) Typical mEPSC recordings in DKO cells rescued with GFP (control), Doc2b^{WT}, or Doc2b^{D218,220N}. Spontaneous mEPSCs were recorded in network cultures of hippocampal neurons in the presence of TTX and gabazine. Increasing concentrations of extracellular Ca²⁺ were administered to the same cell. (B) Mean mEPSC frequencies ± sem in DKO cells expressing Doc2b^{WT} (*n* = 10), Doc2b^{D218,220N} (*n* = 26) or GFP as a control (*n* =

21). (C) Mean mEPSC frequencies of the same constructs expressed in wild-type cells. The average mEPSC frequency varied between experiments, but our experimental design prevents confounding effects thereof (see fig. S2E). (D) Mean mEPSC frequencies in DKO cells expressing Doc2b^{WT} (*n* = 35) or mutant Doc2b^{K237,319E} (*n* = 31). (E) Ca²⁺-dependent liposome-binding by the isolated C2A domain of Doc2b^{WT} and Doc2b^{D218,220N} (*n* = 6). ****P* < 0.005; ***P* < 0.01, **P* < 0.05.

brane binding and SNARE binding were required for the strong fusion-promoting effect of Doc2b. Indeed, both mutant Doc2b^{4A} impaired in Ca²⁺-dependent phospholipid binding (fig. S8) and mutant Doc2b^{K237,319E} impaired in SNARE binding (Fig. 4A) promoted fusion to a lesser extent than wild-type Doc2b (Fig. 4H).

Ca²⁺ dependence of Doc2b-driven spontaneous release. If Doc2b generates spontaneous events by acting as a Ca²⁺ sensor, then mutagenesis of its Ca²⁺ binding site should affect the Ca²⁺ dependence of spontaneous release. We thus expressed wild-type or mutant Doc2b in DKO neurons in network cultures (10, 11). The Ca²⁺ dependence of spontaneous release was assessed by superfusing neurons with increasing extracellular Ca²⁺ concentrations (from 0.2 to 10 mM) (Fig. 5A). As expected, the spontaneous release rate increased with increasing Ca²⁺ concentrations (Fig. 5B) (11). Mutant Doc2b^{D218,220N}, which mimics a dominant active Ca²⁺-bound state of the C2A domain (19), caused a massive increase in spontaneous release rate, especially at low Ca²⁺ concentrations (Fig. 5, B and C). The Ca²⁺ dependence of spontaneous release was almost completely lost in Doc2b^{D218,220N}-expressing cells. This correlated well with the Ca²⁺-independent binding of the isolated C2A^{D218,220N} or C2AB^{D218,220N} fragment to liposomes (Fig. 5E and fig. S8). DKO neurons expressing wild-type Doc2b had a higher spontaneous release rate at each Ca²⁺ concentration than DKO neurons expressing GFP (Fig. 5B). Even in cells lacking Doc2, we observed more spontaneous release events at higher Ca²⁺ concentrations, albeit less pronounced than in wild-type or rescued cells. Thus, as seen before (Fig. 1), additional high-affinity Ca²⁺ sensors for spontaneous release may exist. At high extracellular Ca²⁺ concentrations (5 to 10 mM), the spontaneous release rate increased in all groups, a release that may be supported by synaptotagmins (11). We also expressed Doc2b^{WT} and Doc2b^{D218,220N} in neurons from wild-type mice. Consistent with the data from DKO neurons, expression of each construct increased the spontaneous release rate. This was most pronounced in Doc2b^{D218,220N}-expressing cells (Fig. 5C). Conversely, overexpression of a loss-of-function mutant, Doc2b^{K237,319E}, caused significantly lower mEPSC frequencies at all Ca²⁺ concentrations compared with Doc2b^{WT} in DKO neurons (Fig. 5D). Because PIP₂ binds to the same region as SNAREs (36), we cannot exclude a contribution of PIP₂ binding in this experiment. However, our in vitro fusion assay was performed in the absence of PIP₂ so here, the profound loss of Doc2b^{K237,319E} function was almost certainly due to its loss of Ca²⁺-dependent SNARE-binding (Fig. 4H). Thus, manipulation of the Ca²⁺ dependence of Doc2b affected the Ca²⁺ dependence of spontaneous release, which implicates Doc2b as a Ca²⁺ sensor.

Conclusions. Here we have found that Doc2b is a high-affinity Ca²⁺ sensor for half (hippocampus) or most (cerebellum) of the spontaneous neurotransmitter release events in CNS synapses. Doc2b acts as a functional synaptotagmin-1 ana-

log, operating at smaller Ca²⁺ increases and with higher in vitro fusion efficiency. In contrast to synaptotagmin-1, which has the appropriate affinity to respond to high Ca²⁺ peaks occurring at release sites, Doc2b requires less than 1 μM Ca²⁺ to enhance fusion. Doc2b is not associated with the synaptic vesicle via a transmembrane domain like synaptotagmins and translocates to membranes in response to Ca²⁺ (34), a quality that would explain why evoked release was unaffected in our study. Nevertheless, it is likely that Doc2 proteins are located not far from release sites because of its interactions with SNARE complexes (Fig. 4A), Munc13 (20, 44), and Munc18 (18). Given the sensitivity of Doc2-dependent spontaneous release to BAPTA chelation (Fig. 1E), the protein must be activated close to a source of Ca²⁺, whether this is a Ca²⁺ channel or an intracellular Ca²⁺ store (12–15).

Spontaneous release events appear to be mechanistically heterogeneous. In the synapses studied here, most spontaneous events are Ca²⁺ dependent and require Doc2. Because the spontaneous release in the absence of Doc2 remained sensitive to BAPTA and prolonged neuronal firing at 5 Hz, one or more additional high-affinity Ca²⁺ sensors probably exist. Finally, BAPTA did not block all spontaneous release events and thus, some events are probably truly Ca²⁺ independent, which may be supported by factors such as synaptotagmin-12 (16).

Because multiple C2 domain-containing proteins are coexpressed in synapses and at least Doc2b and synaptotagmin-1 compete for SNARE complex binding, a certain degree of redundancy probably exists among these protein families. The concentrations of various C2-domain proteins and their Ca²⁺ affinity will determine the Ca²⁺ sensitivity of release and the release probability of vesicles. Differences in this balance may explain the large differences in spontaneous release frequencies observed among neurons. Along the same lines, it is conceivable that the increased frequency of spontaneous release in synaptotagmin-1-deficient mice is triggered by Doc2 or other C2 domain-containing proteins (41, 45, 46) that replace synaptotagmin-1 in its absence, where, effectively, synaptotagmin would appear to clamp fusion (11). Finally, whereas spontaneous vesicle fusion could conceivably have been dependent on SNARE assembly alone, we now show that many of these events require the action of curvature-inducing multiple C2 domain proteins.

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Supporting Online Material

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Figs. S1 to S8
Table S1
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Dark Matter Search Results from the CDMS II Experiment

The CDMS II Collaboration*†

Astrophysical observations indicate that dark matter constitutes most of the mass in our universe, but its nature remains unknown. Over the past decade, the Cryogenic Dark Matter Search (CDMS II) experiment has provided world-leading sensitivity for the direct detection of weakly interacting massive particle (WIMP) dark matter. The final exposure of our low-temperature germanium particle detectors at the Soudan Underground Laboratory yielded two candidate events, with an expected background of 0.9 ± 0.2 events. This is not statistically significant evidence for a WIMP signal. The combined CDMS II data place the strongest constraints on the WIMP-nucleon spin-independent scattering cross section for a wide range of WIMP masses and exclude new parameter space in inelastic dark matter models.

A wide variety of observational evidence (1) indicates that $\sim 85\%$ of the matter in our universe is in some nonluminous form that has thus far eluded laboratory identification. The inferred properties of this dark matter suggest that it is composed of elementary particles beyond those described in the Standard Model of particle physics. weakly interacting massive particles (WIMPs) (2) are a class of candidates to constitute this dark matter that are particularly well-motivated by independent considerations of cosmology and particle physics (3–5). If WIMPs constitute the dark matter in our galaxy, they should occasionally scatter elastically off atomic nuclei in a terrestrial target (6, 7). Laboratory searches for such scattering events (8–10) establish their rate to be less than 0.1 per day per kilogram of target mass, and researchers have begun to test the most interesting classes of WIMP models.

The Cryogenic Dark Matter Search (CDMS II) experiment seeks to detect recoiling atomic nuclei (nuclear recoils) from WIMP-scattering events using particle detectors operated at cryogenic temperatures (<50 mK) (8, 11). Each detector is a semiconductor disk ~ 10 mm thick and 76 mm in diameter, which is photolithographically patterned with sensors to detect the phonons and ionization generated by incident particles. These detectors have extraordinary power to distinguish nuclear recoils (produced by interactions of WIMPs or neutrons) from the far more common electron recoils produced by incident photons and electrons. Nuclear recoils generate less ionization than electron recoils of the same deposited energy, allowing event-by-event rejection of electron-recoil events with a misidentification rate of <1 in 10^4 . Electron recoils within a few μm of the detector surface can suffer from reduced ionization collection, but these may be identified by the relatively fast arrival of their

phonon signals. Combining the ratio of ionization to phonon recoil energy (ionization yield) with the timing of the phonon signals gives an overall misidentification rate of <1 in 10^{-6} for electron recoils.

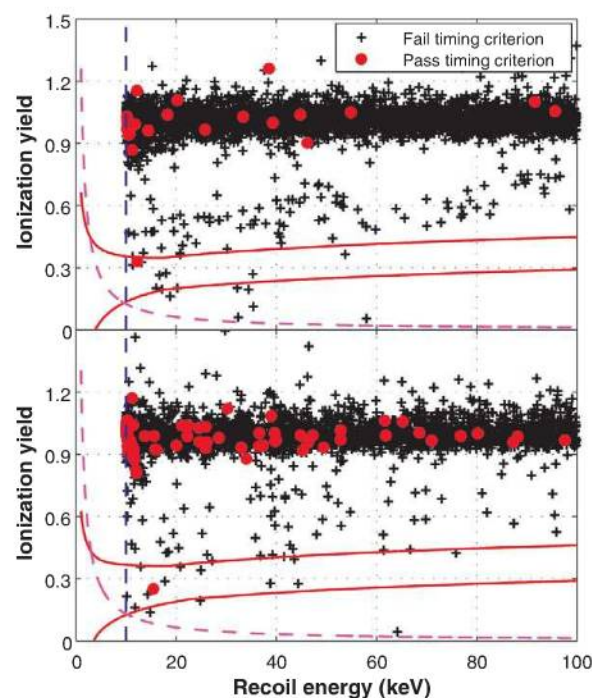
CDMS II operated an array of 30 such detectors (19 Ge and 11 Si) in a low-radioactivity installation in the Soudan Underground Laboratory, Minnesota, USA (11). The depth of the experimental facility (713 m below the surface) greatly reduces the rate of background events from particle showers induced by cosmic rays. Nearly all remaining events from this source were identified using a layer of plastic scintillator surrounding the detector volume. Inner layers of lead and polyethylene further shielded the detectors against environmental radioactivity. Data taken during four periods of stable operation

between July 2007 and September 2008 were analyzed for this work. Because of their greater sensitivity to spin-independent WIMP scattering, only Ge detectors were used to search for WIMP scatters. After excluding periods of poor detector performance, a total exposure to WIMPs of 612 kg-days was considered for this work.

After detector calibration, we defined a series of criteria to identify candidate WIMP-scattering events. WIMP candidates were required to deposit 10 to 100 keV of energy in a single detector, have the ionization and phonon characteristics of a nuclear recoil, and have no identifiable energy deposition in the rest of the array or in the scintillator shield. These criteria are described in more detail in the supporting online material (SOM) text. To avoid unconscious bias, we performed a “blind analysis” in which the exact selection criteria were defined without prior knowledge of the content of the signal region or its vicinity. The fraction of nuclear recoil events accepted by these criteria was measured using a calibration sample of nuclear recoil events induced by a ^{252}Cf source. Despite the great discrimination power of this experiment, a small expected rate of misidentified background events remains. In the exposure considered here, we expected to misclassify 0.8 ± 0.1 (statistical) ± 0.2 (systematic) surface electron recoils as WIMP candidates. We also expect neutrons produced by cosmic rays and radioactivity to generate an average of ~ 0.1 nuclear recoils, which would be indistinguishable from WIMP scatters.

After finalizing all selection criteria, we “unblinded” to examine the contents of the WIMP acceptance region (SOM text). We observed two candidate events at recoil energies of 12.3 keV and 15.5 keV (Figs. 1 and 2). These events

Fig. 1. Ionization yield versus recoil energy for events consistent with all signal criteria, excluding yield and timing. The top (bottom) plot shows events for detector T1Z5 (T3Z4) (see SOM text for detector nomenclature). The solid red lines indicate the ionization yield acceptance region. The vertical dashed line represents the recoil energy threshold, and the sloping magenta dashed line is the ionization threshold. Events with phonon timing characteristics consistent with our selection criteria are shown with round markers. The candidate events are the round markers between the red lines.



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Fig. 2. Normalized ionization yield (number of standard deviations from the mean nuclear recoil yield) versus normalized timing parameter (timing relative to acceptance region) for events consistent with all signal criteria, excluding yield and timing. The top (bottom) plot shows events for detector T1Z5 (T3Z4) (see SOM text for detector nomenclature). Events with phonon timing characteristics consistent with our selection criteria are shown with round markers. The solid red boxes indicate the signal regions. The candidate events are the round markers inside the signal regions. The blue histograms indicate the distributions of calibration neutrons along each axis for the respective detector.

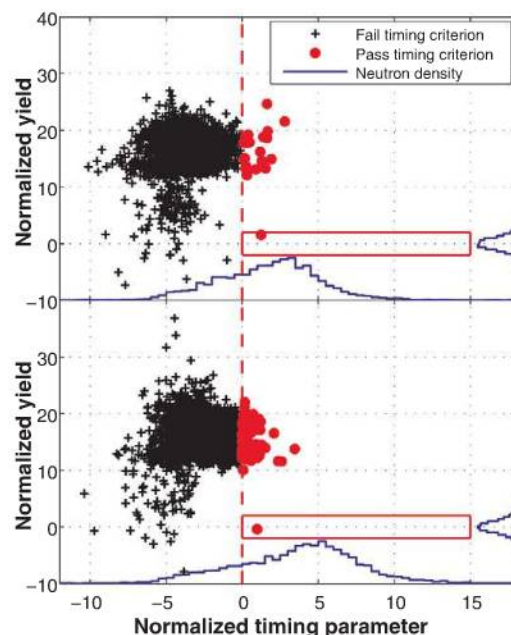


Fig. 3. Experimental upper limits (90% confidence level) and theoretical allowed regions for the WIMP-nucleon spin-independent cross section as a function of WIMP mass. The red (upper) solid line shows the limit obtained from the exposure analyzed in this work. The solid black line shows the combined limit for the full data set recorded at Soudan. The dotted line indicates the expected sensitivity for this exposure based on our estimated background combined with the observed sensitivity of past Soudan data. Previous results from CDMS (8), XENON10 (9), and ZEPLIN III (10) are shown for comparison. The shaded regions indicate allowed parameter space calculated from certain minimal supersymmetric models (17, 18).

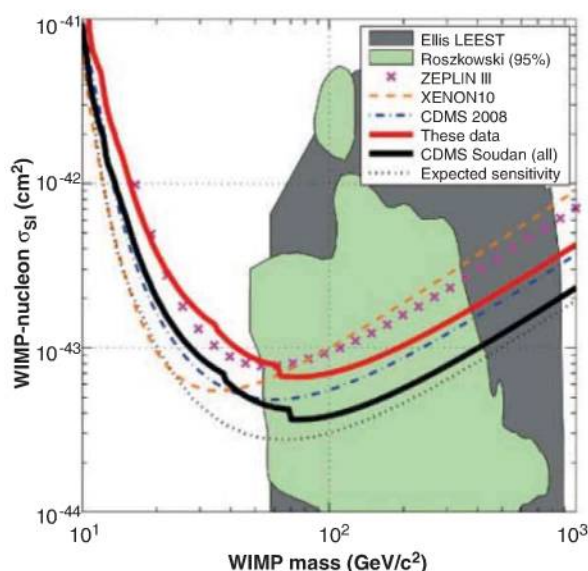
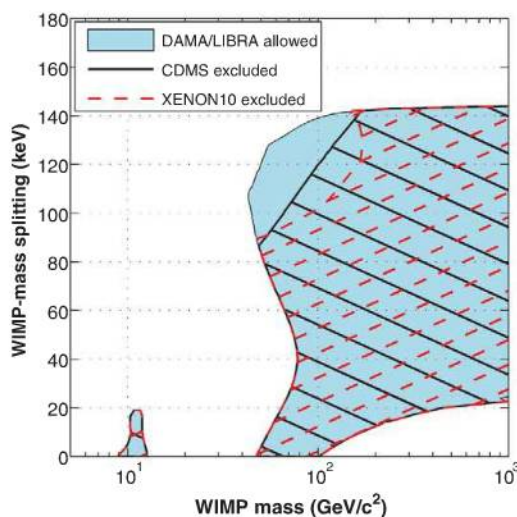


Fig. 4. The shaded blue region represents WIMP masses and mass splittings for which there exists a cross section compatible with the DAMA/LIBRA (14) modulation spectrum at 90% confidence level under the inelastic dark matter interpretation (13). Excluded regions for CDMS II (solid-black hatched) and XENON10 (19) (red-dashed hatched) were calculated in this work using the optimum interval method.



occurred during periods of nearly ideal experimental performance, were separated in time by several months, and took place in different detectors. These candidates match the expectations for WIMP-scattering events, but the probability to have observed two or more background events in this exposure is 23%. The results of this analysis thus cannot be interpreted as significant evidence for WIMP interactions, nor can we reject either event as a WIMP scatter.

These data constrain the spin-independent scattering cross section between WIMPs and nucleons to be $<7.0 \times 10^{-44} \text{ cm}^2$ ($3.8 \times 10^{-44} \text{ cm}^2$ when combined with CDMS II's previous results) for a WIMP of mass $70 \text{ GeV}/c^2$ (12) (Fig. 3). Although this work represents a doubling of our exposure, the observation of two candidate events leaves the combined upper limit nearly unchanged below $60 \text{ GeV}/c^2$ but allows for a modest strengthening in the limit above this mass.

We have also analyzed our data under the hypothesis of WIMP inelastic scattering (13) (Fig. 4). This model has been invoked to reconcile the annual modulation in event rate observed by DAMA/LIBRA (14), with null observations from other experiments. The CDMS II data disfavor all but a narrow region of the parameter space allowed by DAMA/LIBRA that resides at a WIMP mass of $\sim 100 \text{ GeV}/c^2$ and mass splittings of 90 to 140 keV (SOM text).

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12. We calculate the 90% confidence level upper limit based on standard galactic halo assumptions (15) and in the presence of two events at the observed energies. We use the optimum interval method (16) with no background subtraction. A combined limit was also calculated by combining these data with all previous results from Soudan (8), including all candidates and with efficiency weighted by the exposure of each analysis. The abrupt features in these curves are consequences of threshold crossings at which intervals containing one or more events could enter into the optimum interval computation. An improved estimate of our detector masses was used for the reported exposure calculation and applied retroactively to our previous CDMS II result.
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Figs. S1 to S4

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Anomalous Expansion of Attractively Interacting Fermionic Atoms in an Optical Lattice

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The interplay of thermodynamics and quantum correlations can give rise to counterintuitive phenomena in many-body systems. We report on an isentropic effect in a spin mixture of attractively interacting fermionic atoms in an optical lattice. As we adiabatically increase the attraction between the atoms, we observe that the gas expands instead of contracting. This unexpected behavior demonstrates the crucial role of the lattice potential in the thermodynamics of the fermionic Hubbard model.

The striking consequences of correlations in many-body quantum systems are at the frontier of current research. Typically, interest is devoted to the unusual properties of ground states or low-lying excitations, such as exotic types of order and unconventional quasiparticle statistics (1, 2). But correlations can also alter the thermodynamics of a quantum system, leading to fascinating finite-temperature effects. Especially surprising behavior can arise when the system adiabatically enters a strongly correlated phase, as the emerging correlations can imply a substantial redistribution of entropy. A well-known example is the Pomeranchuk effect (3, 4),

which occurs in the liquid-to-solid transition of ³He. As a result of its randomly oriented spins, the solid is more disordered than the liquid. When adiabatically squeezed, the liquid therefore freezes into a solid by absorbing heat.

Recently, the extraordinary progress in the control and manipulation of neutral atoms in optical lattices (5–7) has added a valuable degree of freedom to the investigation of strongly correlated systems. By varying a collection of parameters such as scattering length, lattice depth, and external confinement, it is possible to adiabatically bring a weakly interacting gas of bosonic (7) or fermionic atoms (8–11) into a regime of strong correlations. The versatility of these systems makes them ideal candidates not only to simulate strongly correlated many-body phases, but also to investigate intriguing thermodynamic effects. In particular, the simulation and investigation of the single-band attractive fermionic Hubbard model has received special interest (12–16), both as a means of accessing the preformed-pair or pseudogap regime (12–15) and as an alternative

route to study the physics of the repulsive Hubbard model (16), possibly providing insight into the origin of high-temperature superconductivity in cuprates (17, 18).

We consider an attractively interacting spin mixture of fermionic atoms in two distinct hyperfine states loaded into the lowest band of a three-dimensional (3D) optical lattice and placed in an external harmonic potential. Its physics can be described by a Hubbard Hamiltonian with an additional harmonic confining term:

$$\hat{H} = -t \sum_{\langle \ell, \ell' \rangle \sigma} c_{\ell\sigma}^\dagger c_{\ell'\sigma} + U \sum_{\ell} \hat{n}_{\ell\uparrow} \hat{n}_{\ell\downarrow} + E_c \sum_{\ell\sigma} r_{\ell}^2 \hat{n}_{\ell\sigma} \quad (1)$$

where $c_{\ell\sigma}$, $c_{\ell\sigma}^\dagger$, and $\hat{n}_{\ell\sigma}$ are, respectively, the fermionic annihilation operator, the fermionic creation operator, and the particle number operator at lattice site ℓ (with dimensionless coordinates x , y , and z), and spin state $\sigma \in \{\uparrow, \downarrow\}$, corresponding to the two hyperfine states. We consider the case of an unpolarized system with $N_\sigma = N/2$, where N_σ is the number of particles per spin component and N is the total number of particles. The Hamiltonian (Eq. 1) consists of three competing terms. The first term accounts for the kinetic energy of the system, which is characterized by the hopping amplitude t between neighboring lattice sites; the second term describes the attractive on-site interaction $U < 0$ between atoms with opposite spin (Fig. 1A). The last term represents the confinement energy due to the external anisotropic harmonic potential. The characteristic energy $E_c = V_c r_c^2$ is the mean potential energy per particle and spin state of a maximally packed state at the bottom of the trap, where $r_c^2 d^2$ is the corresponding mean squared radius, $V_c = \frac{1}{2} m \omega_{\perp}^2 d^2$, $\omega_{\perp} = \omega_x = \omega_y = \omega_z / \gamma$ [where $\omega_{x,y}$ are the horizontal trap frequencies and ω_z is the vertical trap frequency], m is the mass of

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an atom, and d is the lattice constant. The squared radius at site ℓ is $r_\ell^2 = (1/r_c^2)(x^2 + y^2 + \gamma^2 z^2)$.

We want to study the size behavior of the system when adiabatically entering the regime of dominating attractive interaction U . To characterize the size, we define the radius R according to

$$R^2 = \frac{1}{N_\sigma} \sum_\ell r_\ell^2 n_\ell \quad (2)$$

where $n_\ell = \langle \hat{n}_{\ell\sigma} \rangle$, the brackets denoting the expectation value at finite temperature. With this definition, the average potential energy per particle and spin state is $E_c R^2$ and the radius of the maximally packed state is equal to 1. As a measure of the change in size in response to an adiabatic change of the interaction strength, we define the interaction compressibility

$$\kappa_i = \frac{\partial R^2}{\partial U} \Big|_S \quad (3)$$

in analogy to the volume compressibility $\kappa_c = -\partial R^2 / \partial E_{c|S}$ (11).

In the experiment, an equal mixture of quantum degenerate fermionic ^{40}K atoms in the two hyperfine states $|F, m_F\rangle = |9/2, -9/2\rangle \equiv |\downarrow\rangle$ and $|9/2, -7/2\rangle \equiv |\uparrow\rangle$ is used. By overlapping two orthogonally propagating laser beams with elliptical shape, a pancake-shaped dipole trap with an aspect ratio $\gamma \approx 4$ is formed. With the use of evaporative cooling in this trap, it is possible to reach temperatures down to $T/T_F = 0.12 \pm 0.03$ (where T_F is the Fermi temperature) with 1.4×10^5 to 1.8×10^5 atoms per spin state. The combination of a red-detuned dipole trap ($\lambda_{\text{dip}} = 1030$ nm) and a blue-detuned optical lattice ($\lambda_{\text{lat}} = 738$ nm) with simple cubic geometry allows an independent control of the confinement energy E_c and the tunneling t . By means of a Feshbach resonance located at 202.1 G (19), the on-site interaction energy U can be tuned at constant tunneling. Negative scattering lengths up to $a = -400a_0$ can be reached; a further approach to the Feshbach resonance is hindered by enhanced losses, heating, and nonadiabatic effects in the lattice (19).

After evaporation, the dipole trap depth is ramped in 100 ms to the desired value of the external confinement ($\omega_\perp = 2\pi \times 20$ to 70 Hz) and the magnetic field is adjusted to set the scattering length. Subsequently, the optical lattice is increased to a potential depth $V_{\text{lat}} = 0$ to $9 E_r$ with a ramp rate of $7 \text{ ms}/E_r$ (19), where $E_r = \hbar^2/(2m\lambda_{\text{lat}}^2)$ is the recoil energy and $\hbar$ is the Planck constant.

We used phase-contrast imaging to extract the cloud size from in situ images taken along the short axis of the trap (19). The experimental data (Fig. 2A) show a contraction of the gas for weak attractive interactions followed by an anomalous expansion for interactions larger than a critical value, which typically corresponds to a scattering length $|a| \approx 20$ to $40 a_0$. Additionally, the fraction of atoms sitting on doubly occupied sites (doublon fraction) is measured via conversion into molecules (11, 19–21), showing a steep increase as the interaction becomes attractive (Fig. 2B). The number of doublons surprisingly continues to increase

as the gas expands, saturating close to 80% for strong interactions and deep lattices. This high doublon fraction, at a density substantially lower than two atoms per site, indicates that the system is in a preformed-pair regime (15). In the absence of the lattice (Fig. 2A), we find that the anomalous expansion disappears [see also (22)] and the size of the cloud remains constant, whereas the doublon fraction can still increase to >40% when the free atom cloud is projected into the lattice.

The theoretical analysis of our results is simplest in the zero tunneling limit, where quantum fluctuations are completely suppressed. In this limit the Hamiltonian is a sum of local Hamiltonians at each site:

$$\hat{h}_\ell = U \hat{n}_{\ell\uparrow} \hat{n}_{\ell\downarrow} + E_c r_\ell^2 (\hat{n}_{\ell\uparrow} + \hat{n}_{\ell\downarrow}) \quad (4)$$

Hence, the problem factorizes into local on-site problems characterized by the probabilities for

Fig. 1. Attractively interacting fermionic spin mixture in an optical lattice. **(A)** The system size R is determined by the interplay among entropy and the different energy scales: the interaction U , the tunneling amplitude t , and the confinement energy. For strong attractive interaction, on-site pairs are formed, which tunnel via second-order processes of amplitude t^2/U . **(B and C)** Schematic illustration of the anomalous expansion using a zero-tunneling two-particle model. The entropy that can be stored per site is reduced as the system evolves from the noninteracting regime, with four possible configurations **(B)**, to the infinitely attractive regime, where particles are tightly bound and only two configurations are available **(C)**. This reduction of entropy per site causes the system to expand [(B) and (C), bottom].

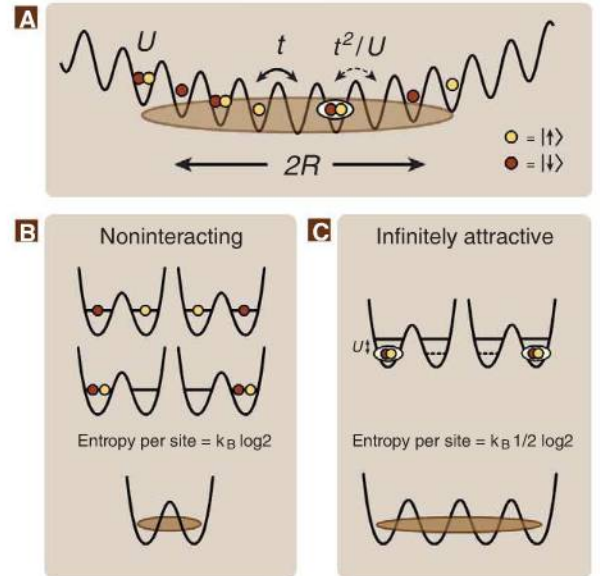
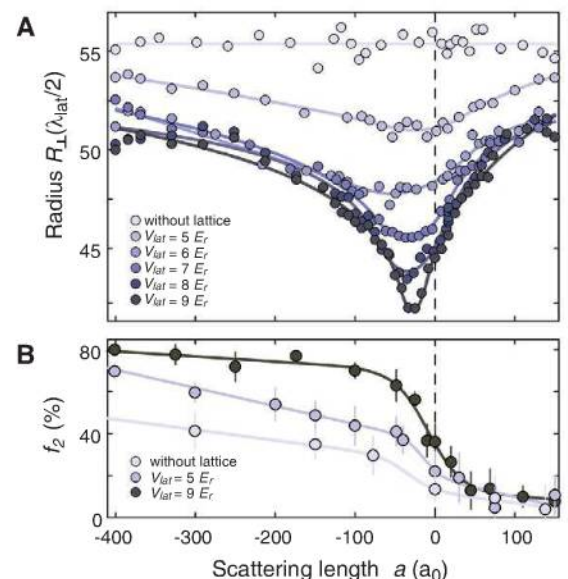


Fig. 2. Experimental observation of anomalous expansion. **(A)** Measured cloud size $R_\perp$ and **(B)** fraction of particles on doubly occupied sites f_2 (doublon fraction) versus scattering length for different lattice depths (5 and $9 E_r$) and in the absence of the lattice. Solid circles in (A) correspond to a running average over three experimental shots. Solid circles in (B) are averages over at least five consecutive measurements, with the standard deviation plotted as the error bar. Lines are guides to the eye. Data were taken in a fixed external dipole trap with $\omega_\perp = 2\pi \times 25$ Hz and aspect ratio $\gamma \approx 4$, at a fixed temperature (before loading of the lattice) of $T/T_F = 0.15 \pm 0.03$ (19).



zero, single, and double site occupation, and the thermodynamic properties can be calculated exactly (19). As interaction increases from zero to infinitely attractive, single occupation is progressively suppressed and the system evolves from a gas of noninteracting fermions with spin, and local entropy $s_\ell = -2[n_\ell \log n_\ell + (1 - n_\ell) \log(1 - n_\ell)]$, to a system of on-site pairs (Fig. 1, B and C). In contrast to pairs in the continuum, which can Bose-condense in the same quantum state, these hard-core bosons occupy lattice sites according to Fermi statistics and have local entropy $s_\ell = -[n_\ell \log n_\ell + (1 - n_\ell) \log(1 - n_\ell)]$, as if they were fermions without spin. The progressive loss of the spin degree of freedom induced by pairing causes a continuous reduction of the entropy density (Fig. 3A). For the same radius (the same n_ℓ), the entropy that can be stored is exactly reduced by a factor of 2. At a fixed entropy, this reduction forces the system to

expand as the attraction increases, exhibiting a negative interaction compressibility κ_i for any nonvanishing attraction and entropy (Fig. 3C).

At finite tunneling, energy minimization and reduction of entropy density compete for the sign of κ_i . For weak attractive interaction, the behavior of the radius is expected to be dominated by the zero-entropy radius, and the system is expected to exhibit a positive κ_i . For a sufficiently

strong attractive interaction, however, the system turns into a gas of hard-core pairs, whose kinetic energy t^2/U vanishes with increasing attraction. The influence of energy then becomes negligible and the reduction of entropy density should dominate, with the system increasing its size and exhibiting a negative κ_i . The value of attractive interaction above which the compressibility becomes negative should increase with tunneling.

Fig. 3. Origin of negative compressibility at zero tunneling. Numerical exact calculation at zero tunneling for a 3D system with $N_\sigma = 7.5 \times 10^3$. **(A)** The squared radius R^2 plotted versus entropy per particle S/N for different attractive interaction strengths, ranging from $U = 0$ (black curve) to $U = -\infty$ (red curve). For a fixed nonvanishing entropy, the radius increases as the attraction increases. At fixed radius, the entropy for the noninteracting gas S^*/N is twice that for the infinite-attraction case. **(B)** Fraction of particles on doubly occupied sites (doublons) and **(C)** radius versus interaction strength at different fixed entropies.

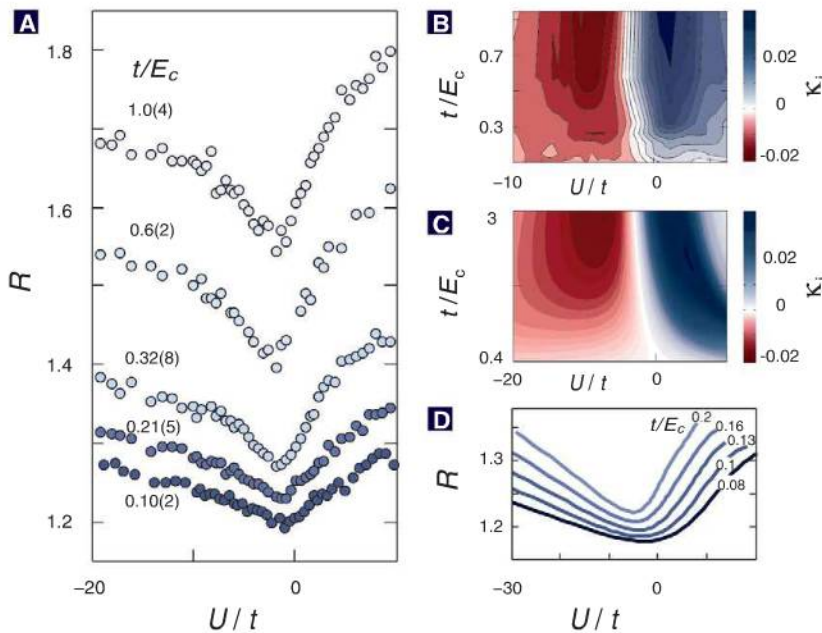
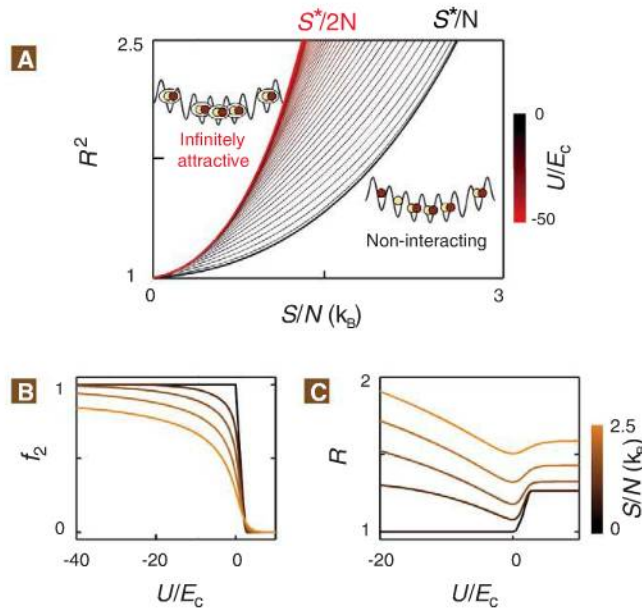


Fig. 4. Comparison of experimental and theoretical size behavior. **(A)** Measured rescaled radius R versus interaction strength U/t for different external confinements t/E_c . Lattice depth is fixed at $7E_r$, and entropy is $S/(k_B N) = 0.9$ to 1.4 ($T/T_F = 0.12 \pm 0.03$). The radius is rescaled in units of the radius of a maximally packed system (see text). **(B)** and **(C)** Experimental **(B)** and calculated **(C)** compressibility κ_i via exact diagonalization of a small system at $S/(k_B N) = 0.7$ (19). Experimental compressibilities are determined from the measured cloud size via a linear fit to 10 consecutive data points. **(D)** Calculated radius of an atom cloud in a 3D lattice using a high-temperature expansion for different external confinements at fixed entropy $S/(k_B N) = 1.6$.

As tunneling increases, the role of interactions is effectively diminished and a larger interaction is required for the entropic effect to overcome the energy minimization effect. The above predictions can be illustrated by exact diagonalization of a small system (19) (figs. S1 and S2). The full many-body problem cannot be solved exactly because of the strong correlations involved. To analyze a 3D many-particle system, we use a high-temperature approximation (19, 23–25) that treats interaction exactly and applies when tunneling is much smaller than either interaction or temperature. The first two terms of the high-temperature expansion capture the competition between energy and entropy and predict a minimum in the cloud radius (Fig. 4D).

In Fig. 4A we show the experimental data obtained at fixed lattice depth for different external confinements, for which the ratios U/t and t/E_c are varied independently. The experimental observation and the theoretical prediction show the same qualitative features (Fig. 4, B and C). For increasing tunneling (decreasing confinement), the observed transition from positive to negative compressibility shifts to stronger attractive interactions. Note that for large ratios of t/E_c , where the role of the external confinement becomes unimportant, the transition occurs at a nearly constant value of U/t —the only energy scale left in the problem.

The size expansion of the gas observed in the experiment when increasing the interaction from zero to the maximum experimental negative value ($|U/t| \approx 20$) is on the order of 5 to 8%. To rule out a possible size increase due to heating, we measured temperatures after reversing the lattice-loading process for all scattering lengths (19). For large values of t/E_c , where the size increase is largest, a very small heating was observed ($\sim 1\%$ of T_F), which could account for only a negligible expansion of the gas (up to $\sim 1\%$), below the experimental shot-to-shot variation.

Our results show how pair formation in an attractively interacting spin mixture of fermionic atoms in an optical lattice gives rise to an anomalous expansion of the gas as the attraction increases. The consequences of pairing in the first band of a lattice potential are fundamentally different from the consequences of pairing in the continuum. Examples of exotic thermodynamic behavior caused by the interplay of strong interactions and entropy have been scarcely observed in quantum many-body systems. We anticipate that such effects can also occur for attractive Fermi mixtures with population imbalance (26), which are currently under investigation. Our work also opens an interesting route toward the detection of quantum many-body phases at finite entropies, where a marked change in the thermodynamic behavior can serve as a footprint of the crossover between two phases exhibiting substantially different entropy densities, as observed recently for a quantum critical system (27). This might be a promising perspective for the detection of transitions between two topological phases

(28), whose different topology can lead to strikingly distinctive ways of storing entropy (29).

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Figs. S1 and S2
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Strontium-Doped Perovskites Rival Platinum Catalysts for Treating NO_x in Simulated Diesel Exhaust

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The high cost and poor thermal durability of current lean nitrogen oxides (NO_x) aftertreatment catalysts are two of the major barriers to widespread adoption of highly fuel-efficient diesel engines. We demonstrated the use of strontium-doped perovskite oxides as efficient platinum substitutes in diesel oxidation (DOC) and lean NO_x trap (LNT) catalysts. The lanthanum-based perovskite catalysts coated on monolith substrates showed excellent activities for the NO oxidation reaction, a critical step that demands heavy usage of platinum in a current diesel aftertreatment system. Under realistic conditions, La_{1-x}Sr_xCoO₃ catalysts achieved higher NO-to-NO₂ conversions than a commercial platinum-based DOC catalyst. Similarly, a La_{0.9}Sr_{0.1}MnO₃-based LNT catalyst achieved NO_x reduction performance comparable to that of a commercial platinum-based counterpart. The results show promise for a considerably lower-cost diesel exhaust treatment system.

There is a recognized need to lower greenhouse gas emissions from mobile sources in order to address concerns regarding global climate change. Diesel engines offer superior fuel efficiency and greenhouse gas reduction potential; however, one of the technical obstacles to their broad implementation is the requirement for a lean nitrogen oxide (NO_x) (NO + NO₂) aftertreatment system, a key contributor to the high cost premium for diesel vehicles. Unlike conventional gasoline engine exhaust, in which equal amounts of oxidants (O₂ and NO_x) and reductants (CO, H₂, and hydrocarbons) are available because of stoichiometric combustion, diesel engine exhaust contains excessive O₂ from combustion with much higher air-to-fuel ratios (>20). This oxygen-rich environment makes the removal

of NO_x much more difficult. A typical diesel aftertreatment system includes a diesel oxidation catalyst, which oxidizes hydrocarbons, CO, and NO, followed by a NO_x reduction catalyst. Two of the most promising technologies for reducing NO_x under the oxygen-rich environment are ammonia selective catalytic reduction (SCR) and lean NO_x trap (LNT). In an SCR system, urea solution is injected into the diesel exhaust and decomposes to form ammonia, which then reacts selectively with NO_x to form N₂ and water. However, urea SCR systems require a secondary fluid tank with an injection system, resulting in added system complexity. Other concerns about urea SCR include urea infrastructure, the potential freezing of urea solution, and the need for drivers to periodically fill the urea solution reservoir. In an LNT-based aftertreatment system, the catalysts contain alkali or alkaline earth components (Ba, K, etc.) that store NO_x in the diesel exhaust to form metal nitrates and nitrites. Once the NO_x storage capacity in the LNT catalyst is saturated, the engine must run rich of stoichiometry (fuel-

rich combustion) to generate excess reductants in the exhaust to remove the stored NO_x on the LNT catalysts and regenerate the NO_x storage capacity. However, the NO_x-absorbing components also react readily with sulfur oxides in the exhaust to form more stable metal sulfates, thus reducing the NO_x storage capacity. Treatment in a reducing environment at high temperatures (>650°C) is required to remove the S from LNT catalysts and recover the NO_x storage capacity.

Many reports have suggested that NO oxidation to NO₂ is an important step in lean NO_x reduction (1–3), because NO₂ enhances the activities of ammonia SCR and LNT. For SCR catalysts, a NO:NO₂ ratio of 1:1 is most effective for NO_x reduction at lower temperatures (<250°C). For LNT catalysts, NO must be oxidized to NO₂ before adsorption on the storage components. Because NO₂ constitutes less than 10% of NO_x in the diesel engine-out exhaust, an oxidation catalyst is required to increase the NO₂ fraction. Platinum has been found to be especially active for NO oxidation; thus, Pt-based diesel oxidation (DOC) and LNT catalysts have been widely used for diesel exhaust aftertreatment (4, 5). However, they suffer from issues such as high cost and poor thermal durability. Consequently, there is substantial interest in the development of better-performing, low-cost, and more durable NO oxidation catalysts.

Here we report that perovskite catalysts show activities for NO oxidation similar to or higher than Pt-based commercial catalysts. Perovskite-based catalysts have been extensively investigated as potential substitutes for precious metal-based catalysts in automotive applications since the 1970s (6–9). The perovskite oxides have a general formula of ABO₃, where A designates a rare-earth or alkaline earth cation and B a transition metal cation (9). They are attractive because of their ease of synthesis, low cost, and high thermal stability (10, 11). The La-based perovskite oxides (such as LaCoO₃, LaMnO₃, and LaFeO₃) have drawn particular interest because their catalytic properties can be easily tuned by substituting a

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small fraction of A-site atoms with other cations such as Ba, Ce, or Sr (11–14). Previous studies have focused on the oxidation of CO and hydrocarbons (9, 12, 13, 15, 16) and NO reduction by CO (17). Despite the extensive research effort, the activities of the perovskite catalysts are typically inferior to those of the precious metal-based counterparts. In addition, like many oxide catalysts, the perovskite catalysts are susceptible to S poisoning (2, 9).

Reports on non-Pt NO oxidation catalysts have been rare. Wen and co-workers reported recently that perovskite catalysts such as $\text{La}_{1-x}\text{Ce}_x\text{CoO}_3$ were more active than Co_3O_4 (14). However, in their work the catalyst activities were measured using a space velocity that was 4 to 5 times lower than that in realistic vehicle exhaust conditions. Furthermore, the effects of S and hydrocarbons were not investigated in their study. Therefore, their results, although interesting, did not provide sufficient information to evaluate the potential of

perovskite catalysts for practical applications such as DOC and LNT.

For the present study, a series of perovskite catalysts including $\text{La}_{1-x}\text{Sr}_x\text{CoO}_3$ ($x = 0, 0.1$) and $\text{La}_{1-x}\text{Sr}_x\text{MnO}_3$ ($x = 0, 0.1$) catalysts were prepared by the citrate method (18). For the LNT catalysts, Pd-Rh/BaO/CeO₂-ZrO₂ catalysts were prepared by a wet impregnation method (19) and then mixed with the perovskite catalyst. The perovskite catalysts were ball-milled, and the slurry was kept at pH = 9.0 by adding aqueous ammonia solution, then wash coated onto cordierite substrates (400 cells per cubic inch). Commercial DOC and LNT catalysts obtained from catalyst manufacturers were used to benchmark the performance of the perovskite-based catalysts. Before the performance evaluation, all the catalyst samples were hydrothermally pretreated in an oven at 750°C for 72 hours to simulate real-world vehicle aging.

Surface areas of the synthesized perovskite oxides were low (7 to 20 m²/g), and they gen-

erally agreed with results reported in previous studies (10). For both LaCoO_3 (11 m²/g) and LaMnO_3 (7 m²/g), the surface areas increased with the addition of Sr (20 m²/g and 15 m²/g, respectively), suggesting that Sr may act as a structural promoter. Surface areas for perovskites are typically low as compared with other oxide-based catalytic materials such as CeO₂ or Co₃O₄, partially as a consequence of the high synthesis temperature (700°C) required for forming a perovskite phase.

Despite the low surface areas, we found the LaCoO_3 and LaMnO_3 catalysts to be highly active for NO oxidation; the NO-to-NO₂ conversions for the LaCoO_3 , $\text{La}_{0.9}\text{Sr}_{0.1}\text{CoO}_3$, LaMnO_3 , and $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ catalysts are plotted as a function of temperature in Fig. 1. Conversion increased with increasing temperatures within the kinetically limited regime and achieved a maximum as the equilibrium limit was reached. LaCoO_3 was more active than LaMnO_3 , and its activity was further enhanced with 10 mole % substitution of Sr, reaching a maximum conversion of 86% at 300°C. In fact, conversions for the $\text{La}_{0.9}\text{Sr}_{0.1}\text{CoO}_3$ catalyst were significantly higher than those for the commercial Pt-based catalyst. The substantial increase in activity by the partial substitution of a La^{3+} ion with a Sr^{2+} ion was also observed by Seiyama *et al.* for hydrocarbon oxidation, and Tanaka and Misono attributed it to a generally accepted explanation that the substitution of Sr greatly increased the amount of weakly bonded oxygen in LaCoO_3 (20). There was no such promotional effect of Sr observed for LaMnO_3 . One possible explanation of this behavior could be the charge imbalance created by the divalent Sr substitution for the trivalent La. Because the highest stable oxidation state for cobalt is +3, this charge imbalance can only be neutralized with O vacancies, which could be associated with the weakly bonded O active for the oxidation reactions. On the other hand, Mn cations can exist in stable +3 or +4 oxidation states, hence the charge imbalance caused by Sr doping in LaMnO_3 could be neutralized by either Mn^{4+} or O vacancies (20, 21). Therefore, doping a small amount of Sr ions in LaMnO_3 may not result in significant O vacancies. However, further investigation is necessary to better understand this difference in the Sr promotion on LaCoO_3 and LaMnO_3 .

In addition to oxidizing NO to NO₂ in the diesel exhaust, a DOC catalyst must also oxidize CO and hydrocarbons. Although the perovskite catalysts are very active for NO oxidation, they are not as active as precious metal-based catalysts for CO and hydrocarbon oxidation. It has been widely reported that Pd [~70% less expensive than Pt (22)] is active for hydrocarbon and CO oxidation (23) but not active for NO oxidation (24). Therefore, a catalyst containing a combination of Pd and perovskite may act similarly to a Pt-containing catalyst.

Another advantage of Pd is that it helps to improve S tolerance of the catalysts. Sulfur is present in most transportation fuels and lubri-

Fig. 1. NO oxidation activities for LaCoO_3 (○), $\text{La}_{0.9}\text{Sr}_{0.1}\text{CoO}_3$ (●), LaMnO_3 (□), $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ (■), and commercial DOC (▲) at a gas hourly space velocity of 30,000 hour⁻¹; 400 parts per million (ppm) of NO and 8% of O₂ in a balance of N₂.

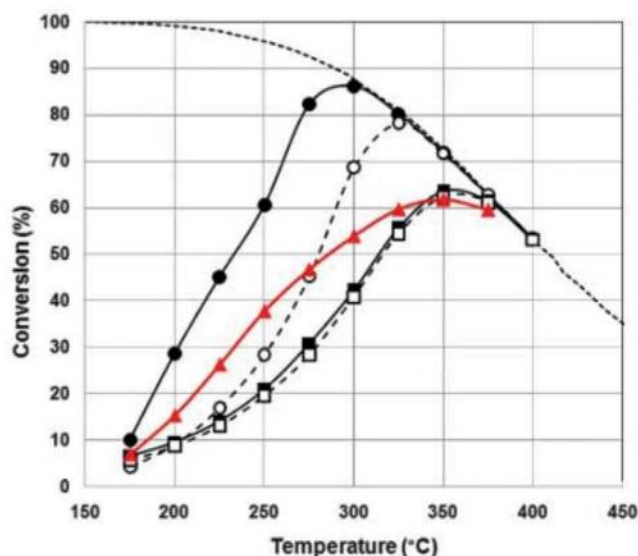
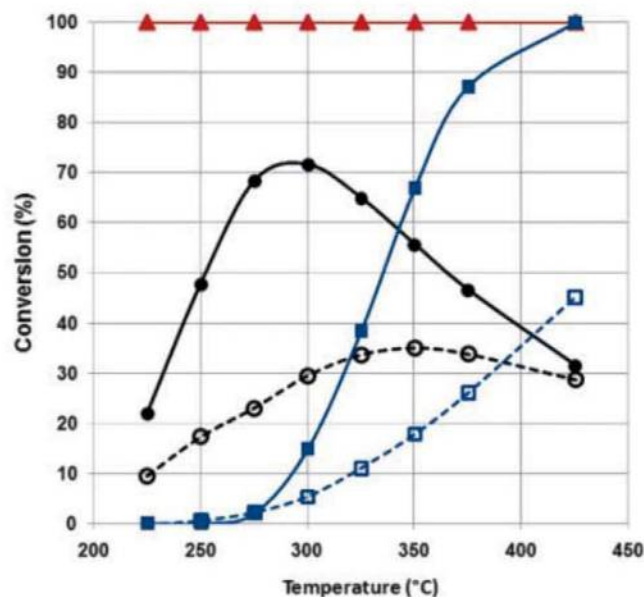


Fig. 2. NO-to-NO₂, C₃H₆, and C₃H₈ conversions under simulated DOC inlet condition after S loading of 1 g liter⁻¹. [C₃H₆] = 245 ppm, [C₃H₈] = 90 ppm, [NO] = 200 ppm, [O₂] = 8%, and [H₂O] = 8% in a balance of N₂. NO-to-NO₂ (●), C₃H₆ (▲), and C₃H₈ (■); commercial DOC (1.6 Pt/0.2 Pt g liter⁻¹) catalyst (dotted line) and 1/2 Pd/Al₂O₃ (1.8 g liter⁻¹) + 1/2 Pd (0.07 g liter⁻¹)/ $\text{La}_{0.9}\text{Sr}_{0.1}\text{CoO}_3$ (solid line).



cants, and the perovskite catalysts are known to form sulfates ($-\text{SO}_4$) when exposed to SO_2 and/or SO_3 , which may lead to collapse of the perovskite structure and loss of catalytic activities. Using a similar strategy to that of Koponen *et al.* (25), we modified our perovskite catalyst with Pd particles. Figure 2 illustrates the oxidation performance of a Pd-only (1.8 g liter^{-1}) catalyst, followed by a Pd/perovskite catalyst compared to a commercial Pt-based catalyst with $1.6 \text{ Pt}/0.2 \text{ Pd}$ (g liter^{-1}) loading after exposure to 1 g of S per liter of catalyst under the simulated diesel exhaust condition. The Pd/perovskite catalyst achieved almost 50% NO -to- NO_2 conversion at 250°C , whereas the commercial catalyst showed less than 20% conversion. In addition, the combination of Pd/ Al_2O_3 and Pd/perovskite catalysts showed similar or better hydrocarbon oxidation performance than that of the commercial DOC catalyst. This Pt-free DOC catalyst thus shows performance and durability superior to those of a commercial Pt-based

DOC catalyst, with a 70% reduction in the metal cost.

In addition to DOC catalysts, the perovskite oxides also show the potential to substitute Pt in LNT catalysts. The importance of NO_2 as an intermediate in the storage of NO_x as nitrates over LNT under O_2 -rich conditions is well documented (24), hence commercial LNT catalysts typically require high Pt loadings. With the excellent NO oxidation activities of the perovskite catalysts, we explored the feasibility of substituting perovskite oxides for Pt in the LNT catalysts. However, using perovskite oxides for LNT applications represents a challenge because of the required frequent exposure to reducing environments during regeneration and/or desulfation processes, which can degrade the perovskite structure. Fierro *et al.* observed such a breakdown of the perovskite structure after H_2 temperature-programmed reduction (TPR) at elevated temperatures (26). H_2 TPR is a useful tool in selecting a durable perovskite LNT catalyst, and Fig. 3 shows

the TPR profiles and powder x-ray diffraction patterns of the $\text{La}_{0.9}\text{Sr}_{0.1}\text{CoO}_3$ and $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ catalysts. The low-temperature H_2 TPR peak below 500°C could be assigned to the removal of non-stoichiometric excess O accommodated within the lattice (27). Cobalt in $\text{La}_{0.9}\text{Sr}_{0.1}\text{CoO}_3$ was completely reduced by H_2 during the TPR, with a concurrent collapse of the perovskite structure (inset) and the formation of discrete oxide phases after re-oxidation in air at 550°C (10, 26); meanwhile, $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ maintained the perovskite structure with no formation of the oxide phases. Therefore, $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ was chosen for LNT applications because of its structural stability (Fig. 3), despite its lower NO oxidation activities (Fig. 2).

NO_x conversions over a $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ -based LNT with precious metal loadings of $1.8 \text{ Pd}/0.2 \text{ Rh}$ (g liter^{-1}) are plotted as a function of temperature in Fig. 4, and NO_x reduction efficiencies were comparable to those of a commercial Pt-based LNT with $1.6 \text{ Pt}/0.3 \text{ Pd}/0.2 \text{ Rh}$ (g liter^{-1}) loading. Over 90% NO_x conversion was achieved at 350°C over the $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ -based LNT, whereas the peak NO_x conversion was reached at 400°C over the commercial LNT catalyst. LNT catalysts require frequent desulfation processes to remove the S absorbed on the Ba sites. Accordingly, we examined the effect of S poisoning and desulfation conditions on a perovskite-based LNT. After the sample was exposed to 1 g of S per liter of catalyst, the perovskite-based LNT lost approximately 10% of its initial NO_x conversion efficiency (Fig. 4). After the desulfation process was performed at 700°C in the presence of 3% CO and 1% H_2 , the NO_x conversion was restored to 90% from 82% at 350°C , suggesting that most of the S was removed.

Oxide-based catalysts have typically shown much lower activities for NO oxidation than Pt-based catalysts under the kinetics-controlled temperature regime (14, 28, 29). The Sr-doped Co- and Mn-based perovskite catalysts reported in the present work showed NO oxidation activities similar to or higher than those of Pt-based catalysts under realistic conditions. The potential use of perovskites for automotive applications is hindered by the fact that the perovskites alone are susceptible to deactivation by S. However, the NO_x -treating performance of Pd/perovskite-based DOC and LNT catalysts in simulated diesel exhaust demonstrated the potential of Pd/perovskite catalysts as a viable substitute for Pt in diesel aftertreatment catalysts. This substitution could drastically reduce the cost of diesel aftertreatment systems for mobile applications. Lean-burn gasoline engines will also benefit from this technology.

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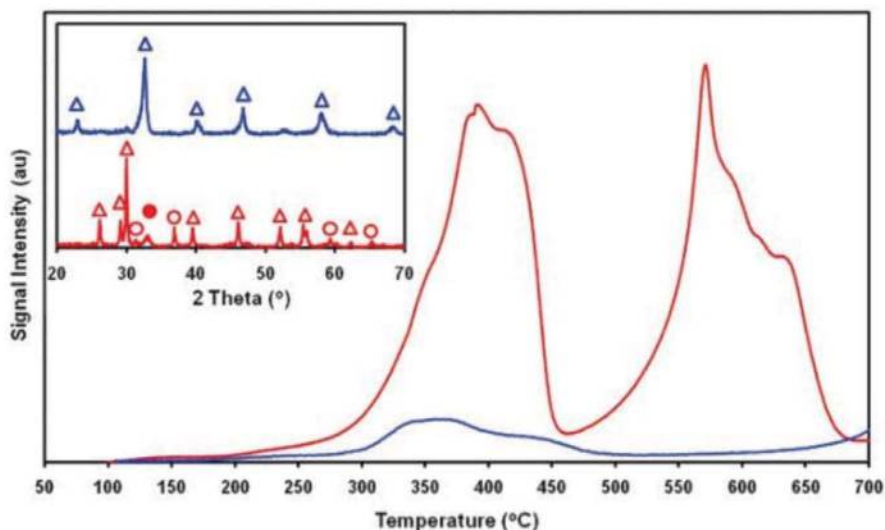
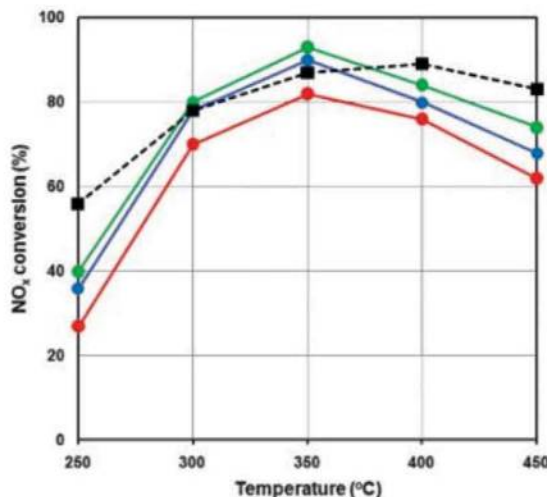


Fig. 3. TPR profiles and x-ray diffraction patterns of $\text{La}_{0.9}\text{Sr}_{0.1}\text{CoO}_3$ (red lines) and $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ (blue lines) reoxidized at 550°C after TPR. Symbols are as follows: $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ (blue Δ); La_2O_3 (red Δ); Co_3O_4 ($\circ$); and LaCoO_3 ($\bullet$).

Fig. 4. NO_x conversion profiles over the commercial LNT (black dashed line), $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ -based LNT (green line), $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ -based LNT after a S loading of 1 g liter^{-1} catalyst (red line), and $\text{La}_{0.9}\text{Sr}_{0.1}\text{MnO}_3$ -based LNT after desulfation (blue line) as a function of temperature. Details of the reaction conditions are described in the supporting online material.



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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S3
Table S1
References

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Heme-Like Coordination Chemistry Within Nanoporous Molecular Crystals

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Crystal engineering of nanoporous structures has not yet exploited the heme motif so widely found in proteins. Here, we report that a derivative of iron phthalocyanine, a close analog of heme, forms millimeter-scale molecular crystals that contain large interconnected voids (8 cubic nanometers), defined by a cubic assembly of six phthalocyanines. Rapid ligand exchange is achieved within these phthalocyanine nanoporous crystals by single-crystal-to-single-crystal (SCSC) transformations. Differentiation of the binding sites, similar to that which occurs in hemoproteins, is achieved so that monodentate ligands add preferentially to the axial binding site within the cubic assembly, whereas bidentate ligands selectively bind to the opposite axial site to link the cubic assemblies. These bidentate ligands act as molecular wall ties to prevent the collapse of the molecular crystal during the removal of solvent. The resulting crystals possess high surface areas (850 to 1000 square meters per gram) and bind N₂ at the equivalent of the heme distal site through a SCSC process characterized by x-ray crystallography.

In biological environments, hemoproteins perform many important chemical tasks involving diatomic gases (1). These include oxidative catalysis by activating molecular oxygen under mild conditions (e.g., cytochrome P450), the storage and transport of oxygen (e.g., hemoglobin and myoglobin), and sensing of gas molecules such as CO and NO (e.g., guanylyl cyclase). Increasingly sophisticated synthetic models of the iron porphyrin in the heme reactive site have been used to understand this complex coordination chemistry and to prepare biomimetic catalysts (2). These model systems share at least some of the following structural features with the heme reactive site: an iron porphyrin, the differentiation of the two axial binding sites (proximal and distal), a proximal ligand to control gas binding, and steric protection of the distal binding site to prevent μ -oxo dimer formation. These synthetic models are

designed to function in homogeneous solution, generally organic solvents rather than the environmentally benign aqueous media of hemoproteins.

Here, we demonstrate a heme model system based on a nanoporous crystal that possesses the basic requirements of these homogeneous models but with the added advantage that axial coordination chemistry at the iron cation is achieved by single-crystal-to-single-crystal (SCSC) transformations that can be characterized directly by x-ray diffraction. Furthermore, these crystals are compatible with aqueous media, and their nanoporous structure ensures rapid access of ligands to the axial binding sites, even by simple adsorption from the gas phase. Ultimately, the added convenience of these nanoporous crystals could lead to the development of practical industrial catalysts. In addition to Fe²⁺, we show that other transition metal cations (e.g., Co²⁺, Mn²⁺, and Ru²⁺) that are known to impart useful catalytic activity to porphyrins and phthalocyanines are incorporated successfully into the nanoporous molecular crystal (3).

Progress in the development of crystalline nanoporous materials (4–6), such as the metal-organic frameworks (MOFs), has produced numerous examples incorporating a metalporphyrin

component (7–17). However, despite the obvious motivation to prepare nanoporous heme models, no such material containing an iron porphyrin has emerged. It is likely that the high reactivity of the Fe²⁺ cation and its strong preference to be hexacoordinate results in competitive ligand binding that interferes with MOF formation. We reasoned that a nanoporous molecular crystal derived from an appropriate metallomacrocyclic by simple crystallization would avoid this problem. Furthermore, the subsequent study of coordination chemistry at the axial sites of the Fe²⁺ cation would benefit from the absence of an extended framework containing transition metals, which could provide competitive binding sites for ligands. In this context, our interest was aroused by the serendipitous discovery of the molecular crystal of zinc 2,3,9,10,16,17,23,24-octa(2',6'-di-*iso*-propylphenoxy)phthalocyanine, which contains very large (8 nm³) solvent-filled voids (18). The phthalocyanine macrocycle (i.e., tetraazatetra-benzoporphyrin) is closely related to porphyrin, and, importantly, the coordination chemistry of iron phthalocyanine is very similar to that of iron porphyrin (19). Hence, we prepared the analogous Fe²⁺ complex (Fig. 1) from 4,5-(2',6'-di-*iso*-propylphenoxy)phthalonitrile and ferrous acetate by using a well-established synthetic procedure (20). Pleasingly, this complex gave a phthalocyanine nanoporous crystal (PNC), isomorphous with that of the Zn²⁺ complex (i.e., cubic symmetry, space group = *Pn* $\bar{3}$ *n*, *a* = 3.74 ± 0.03 nm, *Z* = 12), on diffusion of methanol into a concentrated chloroform solution. Even minor structural modifications to such molecules can alter markedly the subsequent packing arrangement within a molecular crystal. Therefore, it is remarkable that the PNC structure is obtained for many different metal complexes of this particular phthalocyanine derivative (Fig. 1A; M = Mg²⁺, Al³⁺, Ti⁴⁺, Mn²⁺, Fe²⁺, Co²⁺, Zn²⁺, Ru²⁺, or In³⁺), even as they encompass great variation in size, shape, type, and number of axial ligands (Fig. 1A; L). Indeed, the addition of the bulky ligand, *t*-butyl isocyanide (BuNC), to the Fe²⁺ (or Ru²⁺) complex before crystallization enhances PNC formation so that large crystals of several mm in

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diameter can be grown routinely (Fig. 1B). Therefore, we were able to perform and characterize SCSC transformations by the manipulation of single crystals.

The nanoporous structure of the PNCs (Figs. 2 and 3) resembles Schoen's I-WP triply periodic minimal surface (21) in which free volume is unequally partitioned between two interpenetrating labyrinths by a non-self-intersecting, two-sided surface. The larger labyrinth is here termed "void" and is composed of the 8-nm³ voids inside the cubic assembly of six phthalocyanines and the interconnecting channels located at each corner of the assembly; the smaller labyrinth is termed

"cavity" and is composed of the narrow interconnecting channels that lie between the assemblies. For each phthalocyanine complex, the PNC structure differentiates the two axial binding sites so that one faces into the void, here denoted *v*, and the other faces into the cavity, denoted *c*. It is notable that within PNC[cBuNC-Fe-*v*BuNC] the ligand in the relatively narrow cavity (max diameter ~ 1.3 nm) is forced out of axial linearity by steric congestion, whereas the ligand in the more spacious 8-nm³ void is free to adopt a linear configuration (Fig. 3B).

The original solvent of recrystallization within the PNCs [~240 methanol molecules (~20 mass

%) per unit cell, as measured in deuterated chloroform by ¹H nuclear magnetic resonance spectroscopy of the dissolved PNC] can be rapidly and reversibly exchanged with other solvents that do not dissolve the phthalocyanine derivative, such as acetone, hexane, or water, without loss of crystalline order, whereas attempted exchange with toluene, chloroform, or tetrahydrofuran dissolves the PNCs. Rapid exchange of the axial ligands by a SCSC transformation is achieved by the simple addition of a drop of the new ligand to the solvent in contact with a crystal. In each case, the displaced ligand remains in the contact solvent, and the reactions are driven either by a

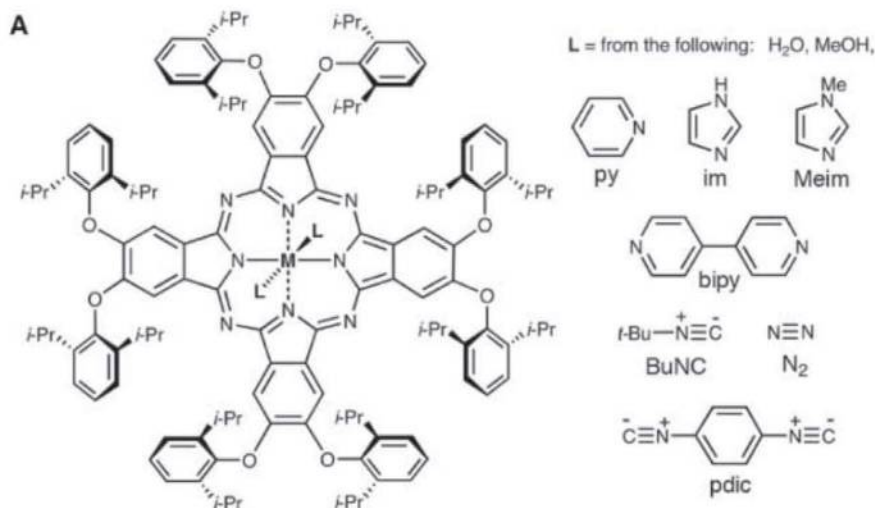


Fig. 1. (A) The molecular structures of the phthalocyanine complexes (*M* = metal cation Mg²⁺, Al³⁺, Ti⁴⁺, Mn²⁺, Fe²⁺, Co²⁺, Zn²⁺, Ru²⁺, or In³⁺) and a selection of axial ligands (*L*) that are compatible with the formation of the

PNCs. (B) Examples of crystals (PNC[cBuNC-Fe-*v*BuNC]) formed by slow diffusion of methanol into a chloroform solution. The cubic crystal on the right is 4 mm in diameter.

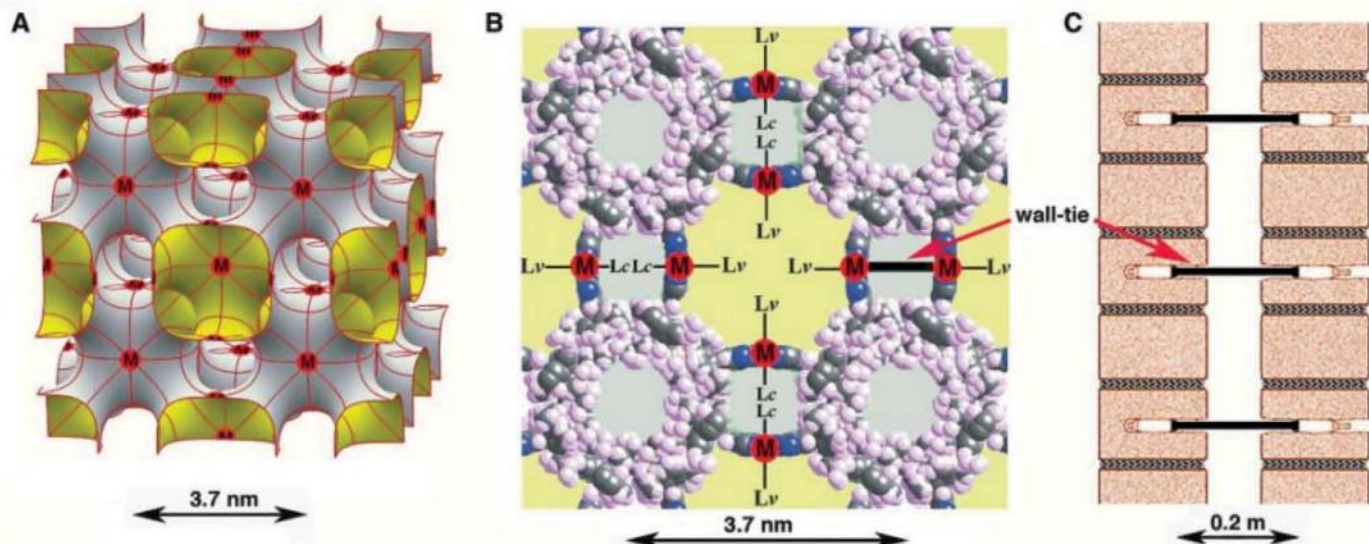


Fig. 2. (A) The nanoporous structure of a PNC as represented by Schoen's I-WP triply periodic minimal surface with the transition metal cations (e.g., Fe²⁺) denoted as *M*. The void side of the surface is shaded yellow, and the cavity side is shaded gray. (B) Schematic representation, based on a space-filling model of the crystal structure, of a cross section through the unit cell of a PNC composed of the phthalocyanine complex where *M* is the

metal cation, *Lv* is the ligand in the void, and *Lc* is the ligand in the cavity. The void enclosed by the hexa-phthalocyanine assemblies is shaded yellow, and the cavity between these assemblies is shaded gray. (C) A cross section through a cavity brick wall showing the role of wall ties in maintaining stability. The location of the analogous bidentate ligand molecular wall tie within the cavity of the PNC is indicated in (B).

higher concentration of the new ligand or by its greater affinity with the metal cation. For example, the BuNC ligand at the void binding site of PNC[cBuNC-Fe-vBuNC] could be partially replaced (70%) by pyridine (py) to give PNC[cBuNC-Fe-v(BuNC)_{0.3}(py)_{0.7}] or quantitatively displaced by *N*-methylimidazole (Meim), with concurrent exchange of the cavity BuNC ligand by methanol originating from the solvent, to give PNC[cMeOH-

Fe-vMeim]. The ligand exchange at the cavity binding site suggested the possibility that bidentate ligands of an appropriate length (~1 nm) might bind simultaneously to two metal cations across the cavity, thus forming a bridge between adjacent hexa-phthalocyanine assemblies. This outcome was achieved with surprising ease by the SCSC addition of either 4,4'-bipyridyl (bipy) or 1,4-phenylenediisocyanide (pdic) to PNC[cBuNC-Fe-

vBuNC] to give PNC[vBuNC-Fe-cbipy-Fe-vBuNC] or PNC[vN₂-Fe-cpdic-Fe-vN₂], respectively. The assignment of N₂ as axial ligand in the latter PNC was unexpected but is supported by infrared spectroscopy (figs. S3 and S4) and has firm precedents in the coordination chemistry of ruthenium porphyrins (22, 23). Indeed, the addition of pdic to PNC[cBuNC-Ru-vBuNC] gives the analogous structure, PNC[vN₂-Ru-cpdic-Ru-vN₂]. This ligand likely originates from the cryogenic stream of nitrogen used to maintain the crystals at 150 K during x-ray diffraction (XRD) analysis, replacing the original vBuNC ligand. The following PNCs were prepared to show the generality of SCSC ligand exchange: PNC[Zn-vpy], by addition of pyridine to PNC[Zn-vH₂O]; PNC[cH₂O-Mn-vim], by addition of imidazole to PNC[cH₂O-Mn-vH₂O]; and PNC[vpy-Co-cbipy-Co-vpy], by addition of bipy to PNC[Co-vpy]. That rapid SCSC axial ligand exchange is achieved within the PNCs with much the same ease as solution-based coordination chemistry can be explained by the lack of structural reorganization required to accommodate even large ligands within the void or cavity. Although axial coordination chemistry within some porphyrin-containing MOFs was inferred by their catalytic activity (13, 17), direct structural characterization of axial ligand exchange on a metallomacrocyclic within a MOF or other nanoporous crystal has been elusive.

As is usually observed for solvent-containing molecular crystals (i.e., clathrates), most of the PNCs suffer structural collapse during the rapid loss of included solvent on exposure to the atmosphere, which is evident by the loss of reflectivity from their macroscopic appearance. However, the PNCs that contain the rigid bidentate ligands, bipy or pdic, within the cavity retain their sheen on prolonged exposure to a stream of nitrogen, a process that ensures the removal of included solvent as determined by thermal gravimetric analysis (TGA). XRD analysis confirmed the retention of the crystal structures and shows that for PNC[vBuNC-Fe-cbipy-Fe-vBuNC] the vBuNC ligand is replaced by N₂. Similarly, for PNC[vpy-Co-cbipy-Co-vpy] the removal of included solvent is accompanied by the loss of the pyridine ligand from the void. These phthalocyanine unsolvated nanoporous crystals (PUNCs) are made by two consecutive SCSC transformations (e.g., PNC[cBuNC-Fe-vBuNC] → PNC[vBuNC-Fe-cbipy-Fe-vBuNC] → PUNC[vN₂-Fe-cbipy-Fe-vN₂]), and it is notable that the metal-metal distance across the cavity is reduced during this process (Fig. 3B). This example of crystal engineering to prevent the collapse of the PNC structure during the removal of solvent is closely analogous to the use of wall ties to stabilize cavity brick walls in architectural engineering (Fig. 2C). It should be emphasized that the molecular wall tie bridges two phthalocyanines to form a dimeric complex rather than forming an extended framework; therefore, the PUNCs are still molecular crystals rather than MOFs. It is of interest that both types of molecular wall tie provide a

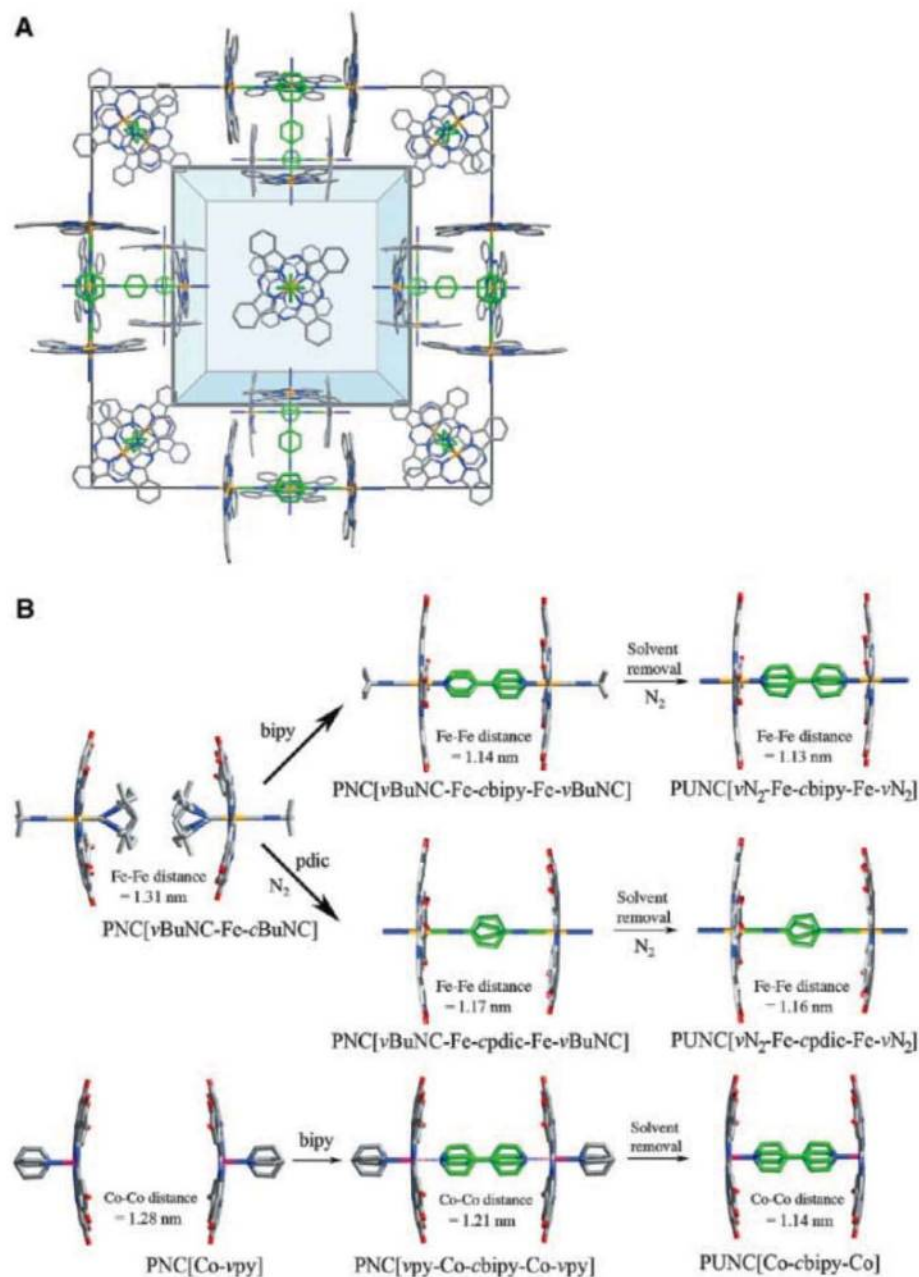
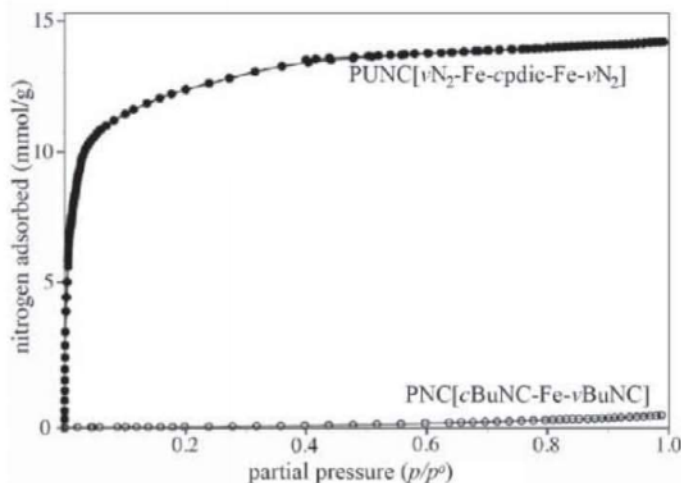


Fig. 3. (A) A perspective representation of the crystal structure of PUNC[vN₂-Fe-cpdic-Fe-vN₂], with the phenoxyl substituents removed and the carbon atoms of the pdic molecular wall ties colored green for clarity. The phenyl ring of the pdic ligand is disordered over two orientations because of the cubic symmetry of the crystal. The large square represents the boundary of the unit cell ($a = 3.7$ nm), and the blue cube represents one of the two evacuated nanovoids, which is located at the center of the unit cell; the other is located in eight 1-nm³ portions at each corner of the unit cell. (B) The SCSC coordination chemistry used to make the PUNCs [vN₂-Fe-cpdic-Fe-vN₂], [vN₂-Fe-cbipy-Fe-vN₂], and [Co-cbipy-Co]. Gray and green, C; blue, N; pink, Co; gold, Fe; and red, O.

Fig. 4. The nitrogen adsorption isotherms of PUNC[$\nu\text{N}_2\text{-Fe-cpdic-Fe-}\nu\text{N}_2$] (solid circles) and an unsolvated (i.e., collapsed) sample of PNC[$\nu\text{BuNC-Fe-cBuNC}$] (open circles) collected at 77 K. A BET surface area of $1002\text{ m}^2\text{ g}^{-1}$ and a total pore volume of 0.46 ml g^{-1} can be calculated for PUNC[$\nu\text{N}_2\text{-Fe-cpdic-Fe-}\nu\text{N}_2$] from these data.



pathway for electronic conjugation between the Fe^{2+} cations that may be useful for the stabilization of mixed oxidation states, which have been implicated in the recently reported low-temperature oxidation of methane by a iron phthalocyanine dimer (24).

The standard test for the confirmation of permanent nanoporosity in a solid is reversible nitrogen adsorption at 77 K following the application of a vacuum to remove adsorbed molecules. The unsolvated crystals of PNC[$\nu\text{BuNC-Fe-}\nu\text{BuNC}$] show minimal nitrogen uptake ($<1\text{ mmol g}^{-1}$) consistent with the prior collapse of structure during removal of solvent (Fig. 4). In contrast, PUNCs [$\nu\text{N}_2\text{-Fe-cbipy-Fe-}\nu\text{N}_2$], [$\nu\text{N}_2\text{-Fe-cpdic-Fe-}\nu\text{N}_2$], and [Co-cbipy-Co] all show significant nitrogen adsorption at low partial pressures (11 to 13 mmol N_2 at $p/p^0 < 0.2$), which confirms the success of the molecular wall-tie strategy in stabilizing these molecular crystals toward the complete evacuation of included molecules. Brunauer, Emmett, Teller (BET) surface areas in the range of 850 to $1000\text{ m}^2\text{ g}^{-1}$ and nanopore volumes in the range of 0.40 to 0.46 ml g^{-1} can be calculated from these isotherms (e.g., Fig. 4). The surface area, total pore volume, and pore size of the PUNCs all exceed those of other nanoporous molecular crystals (25, 26), zeolites (27), and porphyrin-based MOFs (7–17). Nitrogen adsorption studies show that nanoporosity is retained indefinitely under ambient conditions and for samples previously heated to 400 K. For each PUNC, TGA shows a mass reduction at 500 K attributable to the loss of the wall ties, which is likely to coincide with the loss of nanoporosity.

The symmetry-driven self-assembly of the PNCs via crystallization of a phthalocyanine complex, itself made from a symmetry-driven reaction of an easily prepared organic compound, provides a simple and readily scalable (28) method of constructing sophisticated and versatile nanoporous structures. It has been demonstrated that the formation of the PNCs is compatible with a wide selection of transition metal cations, including those that impart useful catalytic activity. It is

highly probable that this range of cations can be extended to many more of the 70 elements, some in different oxidation states, that are known to bind to the phthalocyanine macrocycle (fig. S1) (29). Initial studies show that the related macrocycle 2,3,9,10,16,17,23,24-octa(2',6'-di-*iso*-propylphenoxy)-1,4,8,11,15,18,22,25-octaazaphthalocyanine has very similar PNC-forming properties (30) so that the reactivity of the transition metal center within these materials may also be tuned by modification of the macrocycle structure. Hence, the present study has only begun to explore the scope and potential of the SCSC coordination chemistry that can be performed within the PNCs and PUNCs.

The differentiation of the void and cavity axial binding sites of the phthalocyanine within the PNCs is analogous to the differentiation of the distal and proximal axial sites within hemoproteins (1). Thus, the molecular wall tie within the cavity could perform a similar role to that of a heme proximal ligand (e.g., a histidine or cysteine residue) in the manipulation of the reactivity of the metallomacrocycle in addition to its task of maintaining the structural stability of the molecular crystal. The observation by XRD analysis of a diatomic gas molecule (N_2) at the distal axial site within the PUNCs supports the analogy. This differentiation of the two axial sites by crystal engineering is achieved without the considerable effort that is required to prepare synthetic heme models with differentiated binding sites for use in homogeneous organic solutions (2). Following the recent XRD characterization of a transient reaction intermediate in a MOF (31), the PNCs offer the opportunity to probe intermediates by using similar in situ XRD experiments for a range of metal-centered catalytic reactions, including those of heme proteins. An additional attractive feature of the PNCs for such studies is their long-term hydrolytic stability following the exchange of included organic solvent for water, which will enhance the similarity with the natural environment of hemoproteins and may lead to practical heterogeneous catalysts for use in aqueous media.

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28. It is anticipated that large-scale preparation of octa(2',6'-di-*iso*-propylphenoxy)phthalocyanine and its metal complexes could be achieved readily by processes similar to those that produce many thousands of tons of phthalocyanine derivatives per annum for use as colorants, electronic materials, and homogeneous catalysts for the desulfurization of crude petroleum (29). In addition, we have found that multigram batches of microcrystalline PUNC can be obtained by rapid recrystallization of the phthalocyanine complex in the presence of the bidentate ligand.
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32. This work was supported by Engineering and Physical Sciences Research Council grant no. GR/F019114. We thank F. Tuna (University of Manchester) for help with the synthesis of PNC[Co-cPy], H. Nowell (Diamond) and B. Kariuki (Cardiff University) for technical support during crystallographic data collection, and K. D. M. Harris and G. J. Hutchings for valuable discussions. Crystallographic parameters for the PNCs and PUNCs are available free of charge from the Cambridge Crystallographic Data Centre (CCDC) under deposition numbers CCDC 761407 to 761422.

Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5973/1627/DC1
Materials and Methods
Figs. S1 to S5
References and Notes

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Efficient Annealing of Radiation Damage Near Grain Boundaries via Interstitial Emission

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Although grain boundaries can serve as effective sinks for radiation-induced defects such as interstitials and vacancies, the atomistic mechanisms leading to this enhanced tolerance are still not well understood. With the use of three atomistic simulation methods, we investigated defect–grain boundary interaction mechanisms in copper from picosecond to microsecond time scales. We found that grain boundaries have a surprising “loading-unloading” effect. Upon irradiation, interstitials are loaded into the boundary, which then acts as a source, emitting interstitials to annihilate vacancies in the bulk. This unexpected recombination mechanism has a much lower energy barrier than conventional vacancy diffusion and is efficient for annihilating immobile vacancies in the nearby bulk, resulting in self-healing of the radiation-induced damage.

Designing nuclear materials that can sustain extreme amounts of damage is an important challenge for future nuclear reactors. During service, radiation-induced point defects (interstitials and vacancies) are created (1), which can aggregate to form interstitial clusters, stacking fault tetrahedra, and voids (2–11). Eventually, this can lead to swelling, hardening, amorphization, and embrittlement, which are primary causes of material failure (10–13). It has been speculated that grain boundaries (GBs) and interfaces may serve as effective sinks for defects (14–21). Recently, several nanocrystalline (NC) materials containing a large fraction of GBs [such as nickel (18), copper (18), gold (19), palladium (20), ZrO₂ (20), and MgGa₂O₄ (21)] have been shown to have improved radiation resistance compared with their polycrystalline counterparts. These experimental results are consistent with the expectation that GBs absorb defects, but how defects interact with GBs at the atomic scale is far from understood. In particular, the temperatures in some irradiation experiments are so low [for instance, 100 K (19)] that vacancies are essentially immobile [a vacancy in gold with a barrier of 0.6 eV moves one nearest neighbor distance (2.9 Å) on a time scale of 10¹⁹ s at 100 K], but their annihilation is still somehow enhanced by GBs (19–21). Thus, there must be an alternative mechanism assisting the annihilation of vacancies within grain interiors.

Radiation damage spans from the atomic (nanometer, picosecond) to the macroscopic (meter, year) length and time scales (10, 22). Critical processes involving individual point-defect migration are difficult to observe experimentally (22, 23). Molecular dynamics (MD)

simulations are widely used for simulating defect production (6–8, 14–16). Because of time-scale limitations, only information on the picosecond to nanosecond time scale can be obtained, as longer time scales involving defect annihilation and accumulation are inaccessible. Some high-level modeling methods, including kinetic Monte Carlo (22) and dislocation dynamics (10), are useful for extending time and length scales but require prior knowledge such as defect migration mechanisms and barriers. In complex systems, many key events are non-intuitive (24–26), and neglecting them could result in inaccurate predictions.

We use three simulation methods (27) to investigate the role of GBs in modifying defect behavior in copper, a widely used model system for face-centered-cubic (fcc) metals in radiation damage research (5–11). Specifically, we use

classical MD for simulating defect production at the picosecond time scale, temperature accelerated dynamics (TAD) (26, 28) to bridge the time-scale gap between defect production and aggregation/annihilation near a GB at the microsecond time scale, and molecular statics to obtain thermodynamic properties of defects near the GB.

We begin by performing MD simulations to investigate cascade-induced defect production near a $\Sigma 11 \langle 110 \rangle \{131\}$ symmetric tilt GB (fig. S1) in fcc copper at 300 K. In this 156,312-atom system, a collision cascade is initiated by giving a primary knock-on atom (PKA) 4 keV of kinetic energy. The distance d between the PKA and the GB is varied between 5 and 34 Å, with the PKA directed toward the GB plane. To obtain good statistics on the number of defects produced, we simulated 15 62-ps cascades at each distance. Figure 1A shows the initial conditions of a cascade with $d = 25$ Å. At 0.5 ps, the cascade reaches its maximum size (Fig. 1B). After ~10 ps, the cascade cools down almost completely. Most displaced atoms find crystal lattice sites, but others are permanently displaced, forming interstitials and vacancies. To allow most interstitials to find the GB, we run the simulation for a total of 62 ps (Fig. 1C). Figure S2 shows the corresponding defect population of Fig. 1C. After the cascade cools, there are three vacancies below and two vacancies above the GB. The absorbed interstitials cause a reconstruction of the GB. To avoid counting rearrangements in the GB as defects, we only count defects in the bulk region (3.8 Å or farther from the GB plane, as defined in fig. S2) to characterize the damage state. In these simulations, vacancies are immobile; even when we evolved the cascade for 342 ps, no vacancy motion was observed (movie S1).

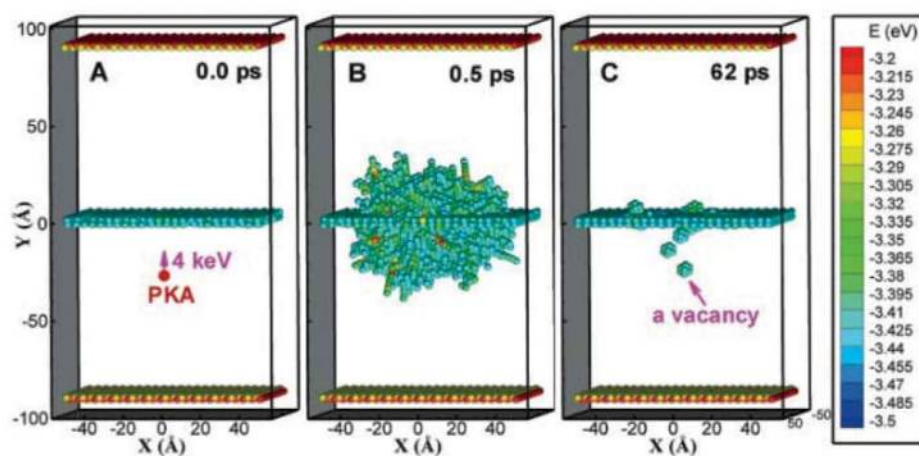


Fig. 1. Representative snapshots of a MD simulation of a collision cascade near a $\Sigma 11$ symmetric tilt GB at 300 K. The atoms are colored by their potential energy; atoms with energies less than 3.43 eV are treated as nondefective and are not shown. The top and bottom layers are fixed surfaces. (A) Initially, a 4-keV PKA is initiated at 25 Å below the GB with its velocity directed perpendicularly toward the GB. (B) After 0.5 ps, the cascade reaches its maximum size. (C) After 62 ps, the cascade cools down with some vacancies remaining below and above the GB. In this display scheme, a vacancy is characterized as a 12-atom cluster, as indicated in (C), because of the increase in energy of the 12 nearest neighboring atoms of the vacancy.

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Figure 2 shows the number of radiation-induced point defects (interstitials and vacancies) in the bulk region as a function of d . The number of vacancies in a single crystal with the PKA matching the orientation and energy of the GB simulations is 9.2 ± 0.8 (SEM) and is shown for comparison. (Note that in the single crystal, the number of vacancies and interstitials are equal.) Clearly, the damage production is sensitive to d . Further, over the entire range of d , the GB preferentially absorbs interstitials over vacancies. Movie S2 shows how this occurs during a cascade. Typically, the number of interstitials in the bulk region is fewer than three, which is substantially less than the average of 9.2 interstitials surviving in the single crystal. In contrast, the number of vacancies in the bulk is either close to or greater than that in the single crystal. As d becomes sufficiently large, we expect that the number of defects produced will approach the bulk value. The number of residual vacancies in the bulk forms a U shape with an optimal distance for maximum defect absorption near 20 Å. Further simulations show that when the PKA energy is increased, the optimum d also increases, implying that the optimal grain size for maximal damage reduction depends on the PKA energy and, thus, the irradiation spectrum.

In the optimum d range, we found that the center of the cascade often overlaps with the GB plane (e.g., Fig. 1B). Because the highest concentration of defects is produced near the center of the cascade, the number of defects in the bulk is reduced the most when the center overlaps with the GB. When the PKA is either closer to or farther away from the GB, the cascade center is either above or below the GB. Because of the biased absorption of interstitials at the GB, a high concentration of immobile vacancies is left in the bulk. In Fig. 2, we also show three snapshots representative of the final defect populations for different PKA distances.

We find that interstitials are loaded into the GB, and many vacancies remain in the nearby bulk during defect production (Fig. 2), consistent with previous studies of random GBs and free surfaces (16, 29). On the MD time scale, this situation remains static (15, 16), but it is interesting to ask how the bulk vacancies evolve over longer time scales (annihilating or aggregating), as this determines the macroscopic response of the material. To investigate this behavior, we use TAD simulations at 300 K to follow the defect kinetics over long time scales with full atomic fidelity.

The initial atomic configuration (Fig. 3A) in our TAD simulations is the damage structure after a 3.0-keV cascade. The smaller PKA energy allows for a smaller atomic system consisting of 4020 atoms (2160 moving atoms), a necessity because of the computational expense of TAD. We recognize that this system size is small for this PKA energy, but the damage generated is representative of the larger systems: interstitials trapped at the GB with vacancies remaining in the bulk region. In the initial damage structure,

there are five vacancies below and two vacancies above the GB, with seven net interstitials absorbed at the GB. The interstitials at the GB form a single cluster, which appears as two in Fig. 3B because of the periodic boundary conditions. Initially, the five vacancies below the GB rearrange with activation barriers (E_a) between 0.16

and 0.42 eV to form a cluster (Fig. 3B). At 23.0 ns (Fig. 3, C and D), an event with $E_a = 0.17$ eV leads to the annihilation of three vacancies, reducing the system energy by 4.90 eV. Comparing the initial and final states of this transition, we find that the vacancy annealing mechanism is very complex. In Fig. 3C, those atoms displaced

Fig. 2. The number of surviving defects in the bulk region as a function of the initial PKA distance d from the GB. Each point is averaged over 15 independent MD runs and is mean $\pm$ SEM (indicated by error bars). The number of vacancies (or interstitials) obtained from a single crystal with the same PKA orientation and energy as in GB simulations is indicated by the horizontal dashed line [9.2 ± 0.8 (SEM)]. The three insets show typical post-cascade defect populations at three PKA distances. Green and red spheres represent interstitials and vacancies, respectively. Depending on the initial PKA distance, the vacancies may preferentially reside above, within, or below the GB. Stacking fault tetrahedra (SFT) are also observed.

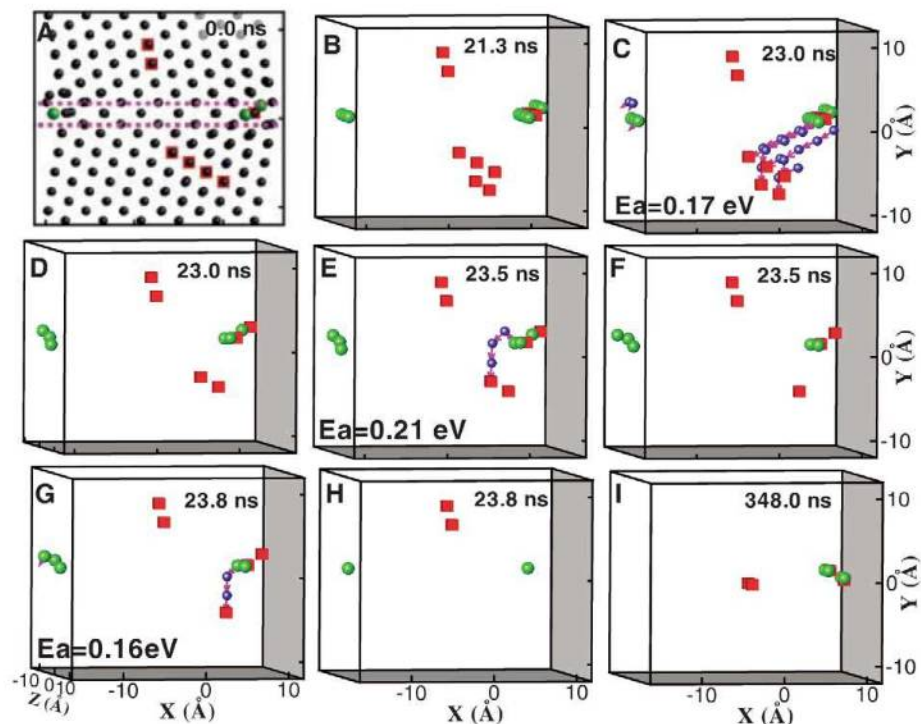
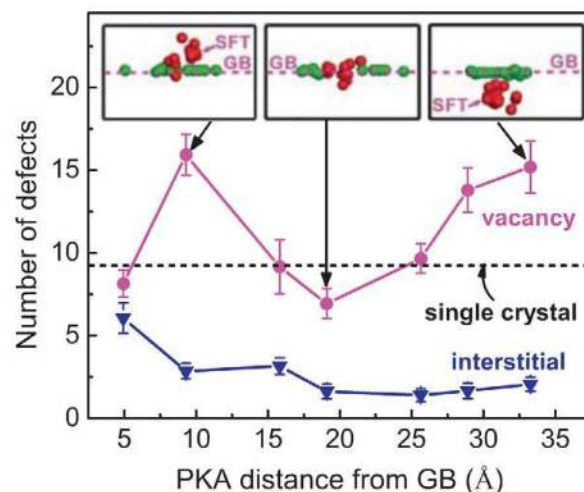


Fig. 3. Selected snapshots of damage self-healing near the GB from TAD simulations. Only the region within 10 Å of the GB is shown. The symbols are defined as follows: smaller black spheres, nondefective atoms [only shown in (A)]; larger green spheres, interstitials; red cubes, vacancies; smaller blue spheres, atoms that move more than 1 Å during an event (between the frames immediately before and after); purple vectors, the moving directions and distances of moving atoms. (A) Initial damage structure produced by MD. The GB region is enclosed by two dashed lines. (B) After 21.3 ns, the five vacancies below the GB form a cluster. (C and D) At 23.0 ns, three interstitials emit from the GB with a barrier of 0.17 eV to annihilate three vacancies. Note that (C) shows how atoms move during this transition, and (D) shows the final configuration after the event is completed. (E and F) Configurations before and after another interstitial emission event at 23.5 ns. (G and H) Configurations before and after the last interstitial emission event at 23.8 ns. (I) At 348.0 ns, the two vacancies above the GB diffuse to the GB via the slower conventional hopping mechanism.

by more than $1.0 \text{ \AA}$ are shown as blue spheres. The most surprising phenomenon is that the interstitial-loaded GB acts as an interstitial source, emitting interstitials via a replacement process, with chains of up to five atoms pushing from the GB to anneal vacancies in the bulk, each atom moving about one nearest neighbor distance. These events occur along $\langle 110 \rangle$ directions, sometimes changing direction to new $\langle 110 \rangle$ directions. We refer to this mechanism as “interstitial emission.” In this mechanism, the vacancies do not move. Instead, interstitials come out from the GB to annihilate bulk vacancies. This is counterintuitive because the interstitial formation energy at the GB is 1.6 eV smaller than in the bulk (fig. S3) and, therefore, the barrier for interstitials to reenter the perfect bulk must be at least 1.6 eV . Thus, interstitials are strongly bound to the GB [supporting online material (SOM) text]. However, when vacancies are present in the nearby bulk, interstitial emission can occur with much smaller barriers (Fig. 3C). Further, nudged elastic band calculations (30) find that the barrier for a vacancy hop in bulk copper is 0.69 eV , much higher than the interstitial emission discovered in this work. Thus, interstitial emission provides a much easier pathway for the GB to annihilate the less-mobile vacancies in the bulk.

After the three vacancies are annihilated, there are two vacancies on each side of the GB (Fig. 3D). The two vacancies below the GB are also annihilated via low-barrier interstitial emission processes (Fig. 3, E to H), after which there are still two vacancies in the bulk and two interstitials in the GB (Fig. 3H). These vacancies form a cluster and diffuse toward the GB via conventional “hopping” (Fig. 3I) with E_a values ranging from 0.23 to 0.39 eV . They finally reach the GB, and the system almost heals itself completely (Fig. 3I); no defects remain in the bulk, though there are still defects trapped at the GB. The system energy decreases $\sim 9.9 \text{ eV}$ relative to the initial state (Fig. 3A). Interstitial emission is not

observed during the hopping of the two vacancies, most likely because the interstitial content of the GB is nearly depleted. Vacancy hopping is not as efficient as interstitial emission for removing vacancies from the bulk; it takes a much longer time (324.2 ns) for them to diffuse to the GB. This disparity in time scales for the two mechanisms would be even greater at lower temperatures. The detailed sequence of the events described above is shown in movie S3. The GB structure before and after annealing is given in fig. S4, showing that the absorbed interstitials lead to a reconstruction of the boundary.

To further investigate the importance of interstitial loading at the GB for annealing vacancies in the bulk, we intentionally loaded the pristine GB with 10 interstitials, which represents a typical damaged structure (27). For both the pristine and loaded GBs, we calculated the vacancy formation energy E_{vac} (27) as a function of the distance from the GB (Fig. 4, A and B). For the pristine GB (Fig. 4A), E_{vac} remains at the bulk value (1.27 eV) until the vacancy is within $\sim 2.0 \text{ \AA}$ of the GB where it decreases only slightly (by 0.2 eV), as compared to the bulk value. However, in the loaded GB (Fig. 4B), E_{vac} changes substantially from the pristine GB: Both the range and strength of the interaction are increased considerably. This is also shown in fig. S5 where the vacancy sites are colored by E_{vac} . The decrease in E_{vac} implies easier vacancy creation and an attraction between the vacancy and the loaded GB. Furthermore, E_{vac} at many sites is less than zero, indicating that the energy of the system actually drops by creating a vacancy. Our analysis shows that a vacancy created at such a site is instantly annihilated upon relaxation via interstitial emission from the GB; thus, it is an unstable vacancy site created by the loaded GB. As shown in table S1, we also find that most vacancies neighboring the unstable sites can be annihilated via interstitial emission with a relatively low barrier. In other words, the range of influence of a

loaded GB extends even further into the bulk from these unstable sites.

To understand how the interstitial-loaded GB affects the vacancy diffusion barrier, we used the nudged elastic band method (30) to calculate the barrier as a function of the distance from the GB for both pristine and loaded GBs (Fig. 4C). Other defect migration barriers in the bulk and near the pristine GB are shown for comparison. For the interstitial-loaded GB, the vacancy diffusion barriers are quite variable because of the complex strain fields induced by the interstitials. Compared with the perfect GB, however, some vacancy diffusion barriers are considerably reduced, and the interaction range is substantially extended (Fig. 4C). In addition, most interstitial emission barriers in the loaded GB are smaller than the vacancy diffusion barriers in the pristine GB. Therefore, interstitial-rich GBs not only induce possible interstitial emission for annihilating bulk vacancies (Fig. 3, C to G), but they also reduce the vacancy diffusion barrier so that vacancies can migrate to the GB more easily (Fig. 3, H and I). These combined effects may explain the enhanced radiation tolerance of materials with GBs.

To assess the generality of our results for other types of GBs, we performed a similar interstitial loading study for an asymmetric $\Sigma 11$ GB, as shown in figs. S6 and S7. The asymmetric GB is a stronger sink for interstitials than the symmetric GB: It has a higher GB energy (0.66 versus 0.31 J/m^2) and a larger decrease in interstitial formation energy (2.6 versus 1.6 eV). Even so, when the asymmetric GB is loaded with 10 interstitials, the vacancy formation energy profile changes considerably, and many unstable vacancy sites are created (fig. S7B), similar to the symmetric GB (Fig. 4B). Further, when vacancies are created near these unstable sites, interstitial emission with low barriers (0.025 to 0.47 eV) occurs (fig. S7C), just as for the symmetric case (table S1). Thus, interstitial emission also

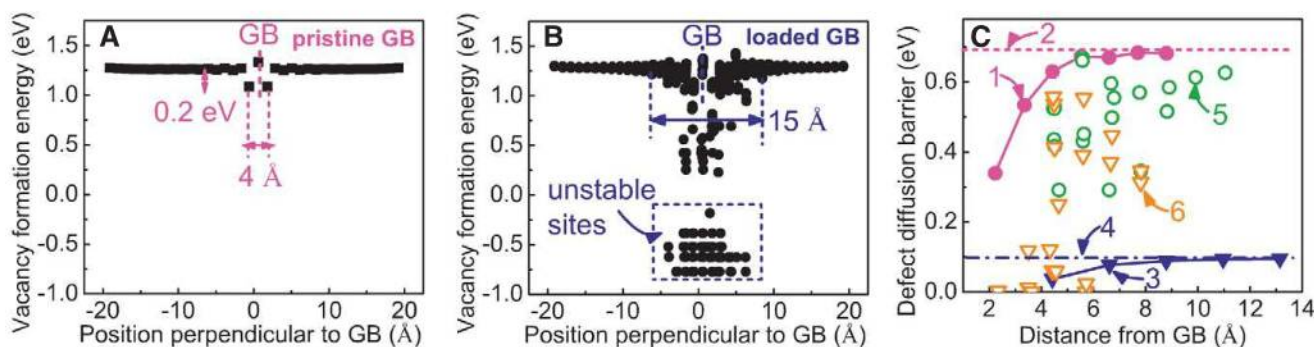


Fig. 4. Influence of interstitial loading on defect properties near the symmetric $\Sigma 11$ GB. (A) Vacancy formation energy profile of a pristine GB. (B) Vacancy formation energy profile of a GB loaded with 10 interstitials, representing the situation occurring after a collision cascade. Upon loading, the GB range of influence expands considerably. Vacancy sites denoted by the dashed box in (B) are unstable sites that are annihilated via barrier-free interstitial emission. (C) Defect diffusion barriers as a function of distance from a pristine and an interstitial-loaded GB. The

symbols and lines are defined as follows: 1, vacancy diffusion barriers near the pristine GB; 2, vacancy diffusion barrier in the bulk; 3, interstitial diffusion barriers near the pristine GB; 4, interstitial diffusion barrier in the bulk; 5, vacancy diffusion barriers near the interstitial-loaded GB; and 6, interstitial emission barriers near the interstitial-loaded GB. Clearly, barriers for vacancy diffusion and interstitial emission near the interstitial-loaded GB are greatly reduced compared with the vacancy diffusion barriers near the pristine GB.

occurs at high-energy asymmetric GBs (SOM text).

Interstitial-rich GBs, typical post-cascade damage structures, play a key role in annihilating the less-mobile vacancies in the nearby bulk via both interstitial emission and enhanced vacancy hopping. Thus, GBs act as efficient sinks for both vacancies and interstitials. The diffusive behavior of defects near a GB is different from the three-dimensional random walk characteristic of defects in the bulk. A GB confines defects such that they are attracted directionally toward the GB. In contrast, the recombination of spatially dispersed defects in the bulk strongly depends on random encounters between them. Once the interstitials are loaded into the GBs, they are spatially localized there and can more readily annihilate the nearby vacancies in the bulk.

The “loading-unloading” mechanism of interstitial emission may help explain the experimental observations that NC materials have better or worse radiation tolerance than polycrystalline (PC) materials, depending on the conditions (18–21). Assuming other types of GBs behave similarly to the two GBs studied here, our results [with the time scales shown in fig. S8 for the three key processes: (i) interstitial diffusion, $E_a \sim 0.1$ eV; (ii) interstitial emission, $E_a \sim 0.2$ eV; and (iii) vacancy diffusion, $E_a \sim 0.69$ eV] explain recent irradiation experiments on NC gold (19), another fcc metal. In that case, at 300 K, the NC form is more radiation-tolerant, whereas at 15 K, the PC form is more tolerant. However, if the 15-K samples are annealed at 100 K, the NC gold recovers more quickly than the PC gold. At 300 K, all three mechanisms are active (fig. S8), so we expect the NC gold to be more radiation tolerant because of the greater concentration of GBs and the ability of vacancies to diffuse to those GBs. At 15 K, if we assume that most of the interstitials have become trapped at the boundary by the end of the hot cascade stage, as we observe in our MD simulations (Fig. 2), then the fact that all thermally activated events are suppressed at 15 K means the excess vacancy concentration in the grain interiors will persist, explaining the reduced radiation tolerance for the NC form relative to PC. When these samples are warmed to 100 K, the interstitial emission becomes active, annihilating vacancies near the GBs, so the NC gold with higher GB density recovers more quickly than PC gold. In ionic materials, such as ZrO_2 (20) and MgGa_2O_4 (21), interstitial emission may be even more important, as defects typically interact over longer distances than in metals, and vacancy migration is even slower.

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Nonepitaxial Growth of Hybrid Core-Shell Nanostructures with Large Lattice Mismatches

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We report a synthetic route to achieving nanoscale heterostructures consisting of a metal core and monocrystalline semiconductor shell with substantial lattice mismatches between them, which cannot be obtained by conventional epitaxial techniques. By controlling soft acid-base coordination reactions between molecular complexes and colloidal nanostructures, we show that chemical thermodynamics can drive nanoscale monocrystalline growth of the semiconductor shell with a lattice structure incommensurate with that of the core. More complex hybrid core-shell structures with azimuthal and radial nanotailoring of structures and compositions of the monocrystalline semiconductor shell are also demonstrated.

Growth of single-crystal semiconductor-based heterostructures with modulated composition is a prerequisite for exploring fundamental nanoscale semiconductor physics (1, 2) and can offer technological devices with optimum characteristics, including enhanced optical properties with high quantum yields (3), engineered electronic bandgaps (4–6), and various solid-state optoelectronic properties (7–9). Unintentional crystalline imperfections (such as polycrystallinity, dislocations, and other structural defects) lead to performance degradation or even premature failure of devices. For example, although the optical quality of semiconductor CdSe

nanoparticles (NPs) could be improved by an overlayer of epitaxially grown CdS or ZnS, problems appear once the shell thickness becomes larger than the critical layer thickness (about two monolayers) due to the existence of strain-induced defects (3, 10, 11). Current methods that achieve high-quality monocrystalline heterostructures are all based on epitaxial growth, which requires moderate lattice mismatches (<2%) between the two different materials. This lattice-matching constraint is a severe obstacle, particularly for growth of core-shell nanostructures with (quasi-) spherical core NPs with highly curved surfaces that present many different crystallographic facets (12). In addition to such lattice-matching requirements, the issues related to differences in crystal structure, bonding, and other properties have been found to inhibit epitaxial growth of dissimilar hybrid materials such as monocrystalline semiconductors on metals (13).

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Attempts to use epitaxy to achieve hybrid core-shell nanostructures have been unsuccessful, resulting in either polycrystalline semiconductor shells or anisotropic structures with segregation of the core and shell, thus limiting their usefulness (14–18). We report a general nonepitaxial growth strategy that achieves precise control of the hybrid core-shell nanostructures, whereby the monocrystalline semiconductor shells are not dependent on the structure of the core NPs. In this approach, growth of the core-shell nanostructures is based on the Lewis acid-base reaction mechanism, where the entire nanostructure is spatially confined by an amorphous matrix (19). Because monocrystalline growth of the semiconductor shell is fully directed by chemical thermodynamic properties of reactions within the matrix, the shell's lattice structure can be independent of that of the core NPs, thus circumventing the limitations imposed by epitaxial strategies.

Figure 1 highlights the results of Au-CdS growth, where the lattice mismatch between the two majority lattice planes of bulk Au and CdS is up to 43% (table S1). Large-scale transmission electron microscope (TEM) images (Fig. 1A) show uniform core-shell nanostructures. The monocrystalline feature of the CdS shell is evident in Fig. 1, B to E. Powder x-ray diffraction (XRD) patterns further confirm that this hybrid core-shell structure grows homogeneously as uniform crystalline domains, and the CdS shells form a hexagonal wurtzite lattice (Fig. 1F). The XRD features of the CdS shells do not show detectable strain-induced bond-length shifts when compared with bulk-indexed peaks, which is different from previous epitaxially grown core-shell nanostructures with much smaller lattice mismatches (20). The perfect crystallinity of the as-grown semiconductor shells is further revealed by angle-dependent TEM characterization under various viewing angles (Figs. 1, G to J). This hybrid Au-CdS nanostructure is stable for months without noticeable changes in the overall structure or degradation of the quality of the semiconductor shell.

The steps of our synthesis protocol are outlined in the flowchart in Fig. 2A. We use Au-CdS as an illustrative example of the process to substantiate our proposed mechanism, in which each growth stage is characterized in detail by high-resolution TEM images (Fig. 2B) and XRD spectroscopy (Fig. 2C), as well as elemental analysis (fig. S1). Our nonepitaxial growth mechanism can be qualitatively understood on the basis of the thermodynamics and coordination chemistry of ionic transformation involved in the growth reactions. Controlling the thermodynamics associated with the chemical transformation processes can initiate and facilitate semiconductor monocrystalline growth in a well-defined amorphous matrix grown outside of the core NPs, and represents the key to our method.

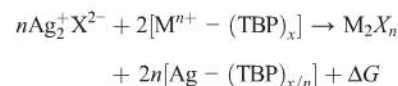
Starting from the core NPs (stage S1), an overlayer of metal with soft Lewis acidity is grown onto the core (stage S2) (19, 21). For all hybrid core-shell structures, we choose a Ag metal overlayer based on the following considerations:

1) According to the theory of hard-soft acids and bases, silver cations behave as a strong acid (acid softness = +3.99) compared with many other common metal cations, such as Zn^{2+} , Pb^{2+} , and Cd^{2+} (fig. S2). Thus, silver cations can easily share their d electrons and coordinate with various soft bases via back-donating π bonds to form a rich family of organometallic complexes. The free energy of reaction (ΔG) is qualitatively determined by the coordination stability of these complexes and can further govern the equilibria of reaction (22, 23). The high acid softness of silver therefore offers broad thermodynamic control of the synthetic process.

2) The silver layer can be grown onto a wide variety of core NPs (including metallic, magnetic, and semiconductor core NPs) with precise thickness control down to a single monolayer (21).

3) The electronegativity of silver is similar to that of many anions X (chalcogenides, As, P) (24). Under certain conditions (e.g., appropriate temperature and anion molecular complexes), the silver shells in stage S2 can be modified to form silver-compound shells (Ag_2X) with an amorphous structure (stage S3) (the amorphous feature of

Ag_2S in stage S3 is confirmed from both high-resolution TEM and XRD patterns shown in Fig. 2, B and C), providing a crucial platform for the next chemical transformation stage, ultimately leading to monocrystalline growth. It has been demonstrated that nanoscale chemical transformations, such as cation exchange, represent a versatile route for converting one crystalline solid to another (25, 26). We show that this process can be harnessed to drive the single-crystal growth by carefully controlling the thermodynamic properties of the reaction (27):



Tributylphosphine was selected because it is a soft base and can behave as a phase-transfer agent to transport metal ions (M^{n+}) to the surface of the core NPs by binding to free cations in solution (fig. S2). The high acid softness of Ag^+ favors the exchange process between Ag^+ in the amorphous matrix and M^{n+} in solution as long as the softness of M^{n+} is small enough to result in a positive ΔG .

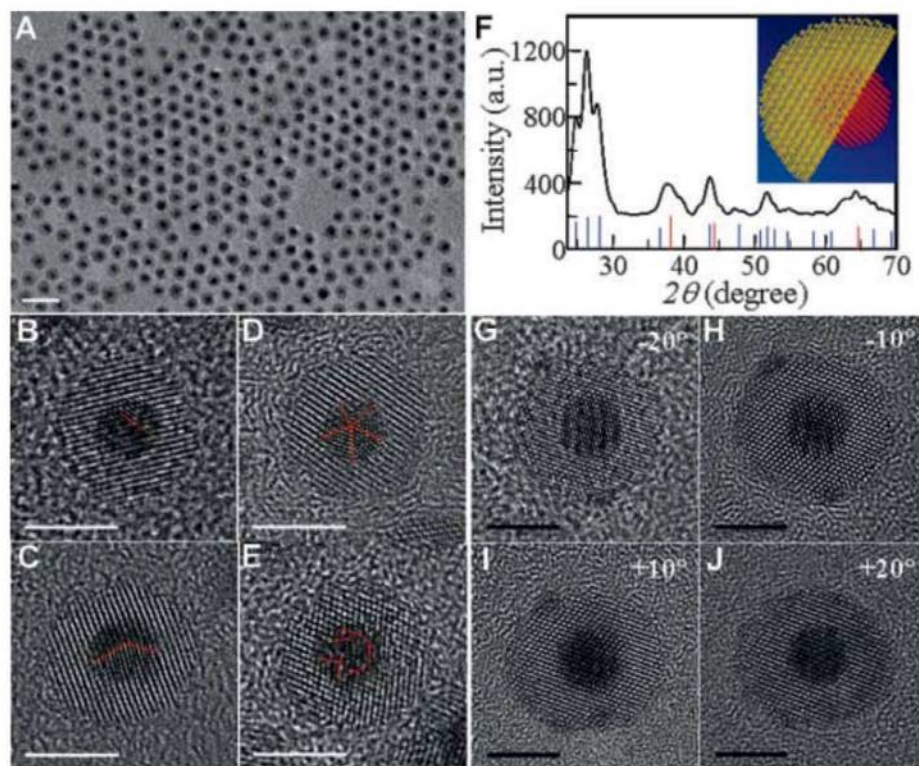


Fig. 1. Au-CdS core-shell nanostructures with monocrystalline shell. (A) Typical TEM image showing uniform core-shell nanostructures. Scale bar, 20 nm. (B to E) High-resolution TEM images of core-shell nanostructures from (A). Whereas Au core NPs can manifest monocrystalline (B), single-fold twin (C), fivefold twin (D), and multiple-twin (E) lattice structures, all CdS shells are monocrystalline. The red lines highlight the lattice orientations within the Au core NPs. Scale bar, 5 nm. (F) XRD pattern of Au-CdS sample shown in (A). Bulk Au [red solid lines, Joint Committee on Powder Diffraction Standards (JCPDS) #04-0784] and wurtzite CdS (blue solid lines, JCPDS #41-1049) are also provided for reference and comparison. (Inset) A ball-and-stick molecular model of Au-CdS, illustrating a cubic core and wurtzite shell. (G to J) Angle-dependent high-resolution TEM characterization. The sample depicted has a larger shell thickness than the one in (A) to emphasize the extremely high-quality crystallinity of the shell. The CdS shell shows perfect monocrystalline features without detectable structural defects under a different viewing angle. Scale bar, 5 nm.

Fig. 2. Nonepitaxial growth process and mechanism of hybrid core-shell nanostructures with substantial lattice mismatches. (A) Schematic of growth process. (B) Series of high-resolution TEM images highlighting different synthetic stages of Au-CdS growth. Scale bar, 5 nm. Red and yellow dashed lines are guides for the eye, distinguishing the core and shell boundaries, respectively. (C) Corresponding XRD patterns of different stages illustrated in (B). Bulk Au (red solid lines, JCPDS #04-0784), monoclinic Ag₂S (green solid lines, JCPDS #14-0072), and wurtzite CdS (blue solid lines, JCPDS #41-1049) are provided for reference. Bulk Ag is not shown because its XRD pattern is very similar to that of Au.

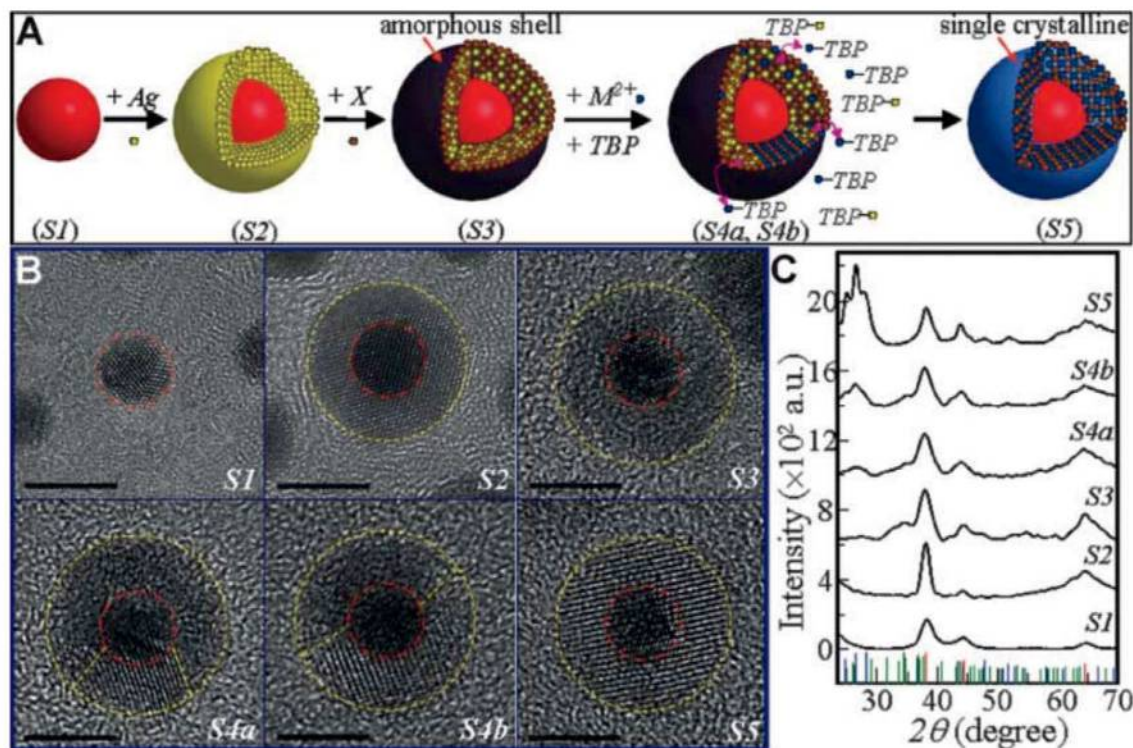
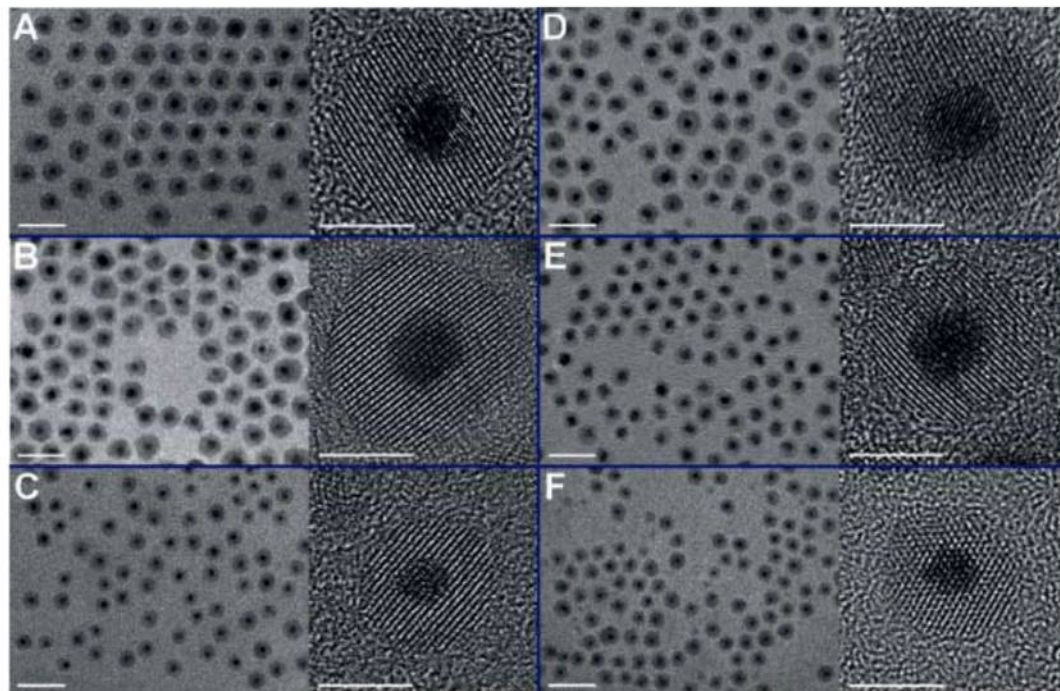


Fig. 3. Large-scale (left) and high-resolution (right) TEM images of different hybrid core-shell nanostructures with various combinations of the core and shell components. All semiconductor shells show monocrystalline features. Scale bars for large-scale and high-resolution TEM images are 20 and 5 nm, respectively. (A) Au-CdSe; (B) Au-CdTe; (C) FePt-CdS; (D) Au-PbS; (E) Au-ZnS; and (F) Pt-CdS.



This in turn provides the impetus to initiate reorganization of the M_2X_n crystalline lattice and to grow into a monocrystalline domain once Ag is completely expelled from the shell (stages S4a-S4b-S5) (fig. S2) (28). The processes from stages S3 to S5 can take from minutes to a few hours depending on the softness of the M^{rt} in solution (19).

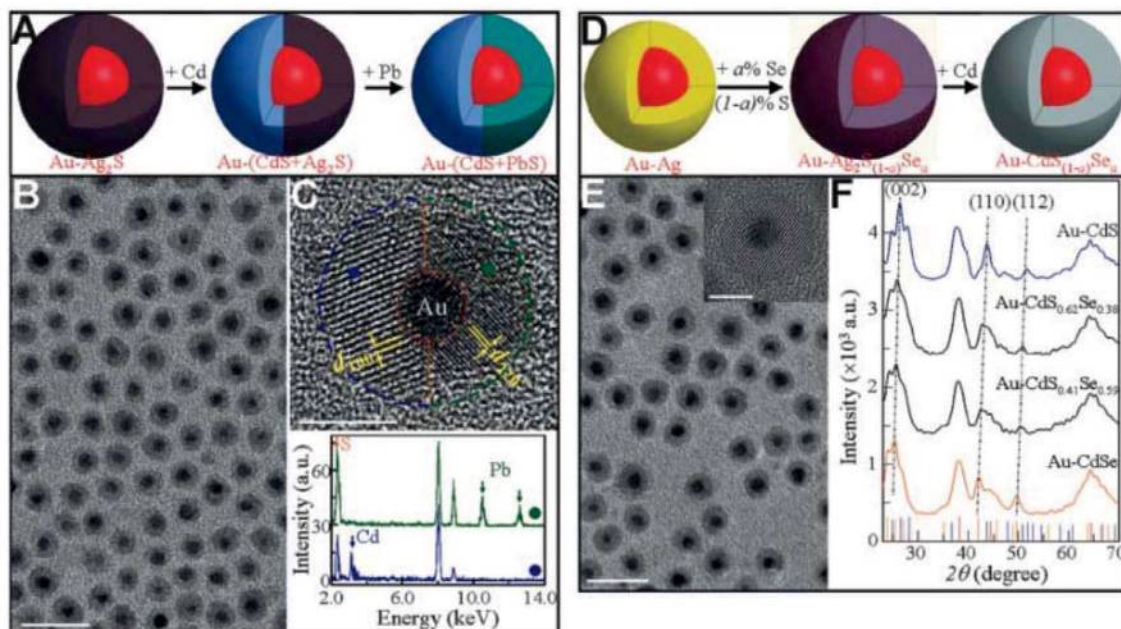
The effect of an amorphous versus crystalline phase of the Ag₂X shell on the resulting crystalline quality, as well as geometry of the core-shell nanostructures, was also investigated. We observed that

crystalline Ag₂S shells typically led to phase segregation between the core and shell, forming non-concentric anisotropic shapes (such as dumbbell nanostructures) (fig. S3). The CdS shells in such asymmetric nanostructures appeared as either polycrystalline or monocrystalline. By contrast, amorphous Ag₂X shells not only provided a well-defined regime for cation exchange (thus defining the dimensions of the monocrystalline semiconductor shells in stage S5), but also promoted the motion of the ions inside the shells as well

as the growth of the monocrystalline domain of M_2X_n due to a reduction of interfacial and grain boundary energies between amorphous Ag₂X and crystalline M_2X_n (28, 29).

According to the above proposed growth mechanism, our technique should be readily applicable to other semiconductor hybrid systems as long as the softness of M^{rt} is less than that of Ag⁺ to achieve positive ΔG (fig. S2). To demonstrate such versatility, Fig. 3 (and figs. S4 and S5) displays different combinations of uniformly grown

Fig. 4. Growth of complex hybrid core-shell nanostructures with tailored structures and compositions of the monocrystalline shells. **(A to C)** Control of the monocrystalline cation species within the shell: the case of Au-(CdS+PbS). **(A)** Schematic of the growth procedure. **(B)** Large-scale TEM image. Scale bar, 20 nm. **(C)** (Top) High-resolution TEM image. Blue and green dashed arc curves highlight the monocrystalline CdS and PbS regimes, respectively. CdS and PbS manifest distinct lattice planes that can be assigned to (100) and (220), respectively. Scale bar, 5 nm. (Bottom) Single-particle EDS measurements in the CdS and PbS regimes. Peaks from Cd, Pb, and S elements are highlighted. **(D to F)** Control of the monocrystalline anion species within the shell: the case of Au-CdS_{1-a}Se_a. **(D)** Schematic growth procedure. **(E)** Large-scale TEM image. Scale bar, 20 nm. (Inset) High-resolution TEM image showing the monocrystalline alloy shell. Scale bar, 5 nm. **(F)** XRD patterns highlighting lattice evolution from CdSe to CdS with different ratio *a*.



hybrid systems using our technique (tables S1 and S2) (19). For all the systems the monocrystalline features of the semiconductor shell, whose lattice structure was determined from XRD measurements (fig. S4), are independent of the core NPs. Similar to the results of Au-CdS, XRD measurements of all hybrid core-shell nanostructures confirm that there is no evidence of strain-induced lattice changes in the semiconductor shell.

One of the important merits of conventional epitaxial growth techniques is precise thickness control. In our nonepitaxial approach, similar precise control of the monocrystalline semiconductor shell layer is achievable because the preceding Ag growth (stage S2) is controllable down to a single monolayer (21). As an example, precise and independent control of the core and shell sizes in Au-CdS is shown in figs. S6 and S7. Because the optical properties of the semiconductor shell and metal core are dependent on their dimensions (due to quantum confinement and surface plasmon resonance effects, respectively), our independent control of both the shell and core dimensions can lead to tunable optical properties, as demonstrated in figs. S6 and S7. An additional advantage of our technique is the clear absence of a critical layer thickness intrinsic in epitaxial growth techniques (1). For instance, the monocrystalline CdS shells were grown up to 15 nm thick onto Au core NPs without detectable structural defects (fig. S8).

Our technique can be used to make more complex nanoscale heterostructures with precise structural and compositional tailoring. Figure 4 (and fig. S9) highlights three examples with independent azimuthal and radial engineering of hybrid core-shell nanostructures. In Fig. 4A, half of the amorphous Ag₂S is first converted into mono-

crystalline CdS shells followed by sequential growth of PbS (this process can be confirmed by monitoring the compositional changes at each stage, as shown in fig. S10). Large-scale TEM images show that this controlled process can preserve the uniformity of the nanostructures (Fig. 4B). High-resolution TEM images reveal two distinct monocrystalline lattices split 50/50 with a Au core in the center, as evidenced by single-particle energy-dispersive x-ray spectroscopy (EDS) measurement (Fig. 4C). Enabled by such, multiple monocrystalline semiconductors can be seamlessly integrated into a single core-shell unit with a precisely tunable ratio of different components (fig. S11).

Whereas Fig. 4, A to C, demonstrate integration of the monocrystalline cation species within the shell, Fig. 4, D to F, illustrate our fine control of anion species. Ternary single-crystal CdS_{1-a}Se_a alloys represent an important semiconductor with a bandgap and lattice constant monotonically tunable by the ratio *a*. They can exhibit large nonlinear susceptibilities, as well as desirable photoconductive properties, and offer promising technological applications, such as a tunable laser (30). Figure 4D schematically shows the procedure for growing a monocrystalline CdS_{1-a}Se_a alloy shell in a typical hybrid core-shell nanostructure, which begins with the reaction of the silver shell formed in stage S2 with a mixture of S and Se organocomplexes (with a predetermined ratio) developing an amorphous Ag₂S_{1-a}Se_a shell followed by sequential cation exchange with Cd²⁺. Figure 4E shows the uniformity as well as monocrystalline features of such an alloy shell grown onto Au core NPs. EDS measurements confirm that the atomic ratio of (S+Se)/Cd is very close to 1, which suggests formation of a ternary phase, but the ratio *a*

is tunable in the monocrystalline shell layer (fig. S12). Powder XRD measurements reveal the lattice evolution of this ternary alloy as a continuous function of ratio *a* from pure wurtzite CdSe to wurtzite CdS; decreasing the S concentration increases the lattice constant of the monocrystalline alloy shell layer.

The excellent stability and monocrystalline quality of the as-synthesized core-shell nanostructures imply that the semiconductor shells can be further applied as a template for continual growth of different shell layers along the radial direction; one such example of hybrid Au-CdS-CdSe core-shell-shell nanostructure is presented in fig. S9. It has been demonstrated that through coupling with surface plasmons in metallic nanostructures, the luminescence intensity of the fluorophores can be significantly enhanced, depending on coupling strength (31–33). Therefore, this radial engineering of the hybrid core-shell-shell nanostructures offers a precise and controllable way to explore such enhancements by tuning the thickness of the CdS shells, and may prove useful for interfacing with biological systems with enhanced bioimaging and biolabeling capability (fig. S13) (19).

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Figs. S1 to S13

Tables S1 and S2

References

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Shaping Development of Autophagy Inhibitors with the Structure of the Lipid Kinase Vps34

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Phosphoinositide 3-kinases (PI3Ks) are lipid kinases with diverse roles in health and disease. The primordial PI3K, Vps34, is present in all eukaryotes and has essential roles in autophagy, membrane trafficking, and cell signaling. We solved the crystal structure of Vps34 at 2.9 angstrom resolution, which revealed a constricted adenine-binding pocket, suggesting the reason that specific inhibitors of this class of PI3K have proven elusive. Both the phosphoinositide-binding loop and the carboxyl-terminal helix of Vps34 mediate catalysis on membranes and suppress futile adenosine triphosphatase cycles. Vps34 appears to alternate between a closed cytosolic form and an open form on the membrane. Structures of Vps34 complexes with a series of inhibitors reveal the reason that an autophagy inhibitor preferentially inhibits Vps34 and underpin the development of new potent and specific Vps34 inhibitors.

The class III phosphoinositide 3-kinase (PI3K), Vps34, is the most ancient paralog of the three classes of PI3Ks in mammals (1). It engages in a wide range of intracellular transport activities, including transport to lysosomes via multivesicular bodies (2), endosome-to-trans-Golgi transport via retromers (3), phagosome maturation (4, 5), and autophagy (6). More recently, signaling roles of Vps34 have been described in nutrient sensing in the mammalian target of rapamycin (mTOR) pathway (7, 8) and signaling downstream of heterotrimeric GTP-binding protein-coupled receptors (9). Given the role of Vps34 in activating mTOR signaling, Vps34 inhibitors could have application in treatment of obesity or insulin resistance (10). One of the obstacles to understanding the cellular

roles of Vps34 is that, currently, there is no inhibitor capable of specifically inhibiting class III PI3K.

Vps34 phosphorylates the D-3 hydroxyl of the phospholipid phosphatidylinositol (PtdIns) to produce PtdIns3P. Proteins containing binding domains such as FYVE or PX that specifically recognize PtdIns3P initiate the assembly of complexes at the membranes of endosomes, phagosomes, or autophagosomes. Vps34 associates with the N-terminally myristoylated, putative Ser/Thr protein kinase Vps15 (hVps15/p150 in humans), which leads to activation of Vps34 (11, 12). Regulatory proteins such as Rab5 and Rab7 bind to Vps15 and enable activation of the Vps34/Vps15 complex at membranes (6, 13, 14).

The Vps34/Vps15 heterodimer is found in multiple complexes in eukaryotes (10), some of which have a fundamental role in autophagy (15). Autophagy has diverse intracellular roles, including degradation of long-lived proteins and organelles and also in maintaining a balance between cell growth and death during development (16, 17). In yeast, Vps15/Vps34/Vps30 form the core of complexes I and II, whereas the

autophagy proteins Atg14 and Vps38 recruit this core for autophagy and endosome-to-TGN (trans-Golgi network) sorting, respectively (18). The mammalian ortholog of Vps30 is Beclin1, which in autophagy associates with hAtg14/Barkor (19, 20) and, in a separate complex, ultraviolet irradiation resistance-associated gene (UVRAG) (21) and Bax-interacting factor-1 (Bif-1) (22). UVRAG has also been proposed to function in endosomal sorting (23).

A construct of *Drosophila melanogaster* Vps34 (DmVps34) lacking the C2 domain (Δ 1-257), referred to as HELCAT (helical and catalytic domains), was used for the 2.9 Å resolution structure determination (Fig. 1A) (24). The C2 domain had no influence on catalytic activity in vitro (figs. S1 and S2), but its role may be to bind Beclin1 (21). The overall fold of the enzyme showed a solenoid helical domain packed against a catalytic domain, forming a compact unit with extensive interdomain contacts (Fig. 1B). The asymmetric unit of the crystals contains a dimer of Vps34 with 1800 Å² of the solvent-accessible surface buried in the interface. The C-terminal helix of one subunit inserts into a prominent slot on the surface of the other subunit (fig. S3). However, light-scattering analyses indicate that Vps34 is a monomer in solution (fig. S4).

One of the most notable features of the Vps34 structure is the completely ordered phosphoinositide-binding or "activation" loop (Fig. 1, B to E, and fig. S5). This loop is critical for the characteristic lipid substrate preferences of the PI3K catalytic subunits (25), but in other PI3K structures, it has been largely disordered (26, 27). The proximal (N-terminal) end of the Vps34 activation loop forms an essential part of the phosphotransferase reaction center (Fig. 1, C to E). The intermediate section forms a vertical wall reaching toward the membrane surface (Fig. 1E). The distal (C-terminal) end of the loop cradles the C-terminal helix from the other molecule in the crystal dimer. Although we have been unable to obtain a Vps34/PtdIns complex structure, it is possible to model phosphoinositide headgroup binding that would facil-

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itate direct transfer of the adenosine triphosphate (ATP) γ -phosphate to the 3-OH of the inositol ring (Fig. 1C). The 1-phosphate of the substrate is likely to be adjacent to the ϵ -amino group of Lys⁷⁷¹-Hs (Lys⁸³³-Dm) at the apex of the loop, which is consistent with our observation that the Lys⁷⁷¹ $\rightarrow$ Ala⁷⁷¹ (K771A) mutant dramatically impairs activity (Fig. 2A). The inositol ring would stack on the hydrophobic surface created by Pro⁷⁷⁰-Hs and Tyr⁷⁶⁴-Hs (832-Dm and 826-Dm) (Fig. 1C). Consistent with this finding, the Tyr⁷⁶⁴ $\rightarrow$ Ala⁷⁶⁴ mutation inactivates the enzyme (Fig. 2A). The D-3 hydroxyl would be in a small pocket lined with catalytic loop residues Asp⁷⁴³-Hs, Arg⁷⁴⁴-Hs, His⁷⁴⁵-Hs, and Asn⁷⁴⁸-Hs (Dm 805-DRHxxN-810). The guanidinium group of Arg⁷⁴⁴-Hs interacts with, and potentially stabilizes, the backbone of the Asp-Phe-Gly (DFG) motif in the activation loop, and the positive charge may also help neutralize negative charge in the transition state of γ -phosphate transfer.

Vps34 residues within the conserved catalytic loop DRH motif (Hs 743-DRH-745 and Dm 805-807) have a conformation that suggests a mechanism whereby His⁷⁴⁵-Hs could act as the catalytic base, abstracting a proton from the

substrate 3-OH to facilitate nucleophilic attack on the γ -phosphate of ATP. Two acidic residues, Asp⁷⁴³-Hs and Asp⁷⁶¹-Hs (Asp⁸⁰⁵-Dm and Asp⁸²³-Dm) are well positioned to act as metal ligands that could help neutralize negative charge in the transition state (Fig. 2B). The p110 γ /ATP structure appears to have captured the catalytic loop in an inactive state in which neither the histidine nor the aspartate of the DRH is properly oriented for catalysis. The difference between the p110 γ and Vps34 catalytic loops may reflect an inactive-to-active transition that is possible for all PI3Ks (Fig. 2C).

An earlier study noted the importance of a C-terminal element for Vps34 activity in vivo (28). The structure shows that this element is part of the C-terminal helix (α 12). This helix has a critical role in catalysis both in vitro (Fig. 2A) and in vivo (Fig. 2D). Truncation of the 10 C-terminal residues of human and yeast Vps34 almost completely abrogates catalytic activity. Even single point mutations in the conserved C-terminal motif Φ Hx Φ xQxWRx greatly reduce enzymatic activity on PtdIns-containing vesicles (Fig. 2A) and in vivo (Fig. 2D). Surprisingly, truncation of the 10 C-terminal residues enhances basal ATPase activity in the absence of lipid

substrate (Fig. 2E). The HsVps34 Trp⁸⁸⁵ $\rightarrow$ Ala⁸⁸⁵ and Tyr⁸⁸⁴ $\rightarrow$ Ala⁸⁸⁴ mutations in the C terminus also increase the basal ATPase activity (Fig. 2E). This suggests that, in the closed form, the C-terminal helix would fold over the catalytic loop locking the catalytic His⁷⁴⁵-Hs (His⁸⁰⁷-Dm) in its inactive conformation (Fig. 3A). In this arrangement, the C-terminal helix would be cradled by the activation loop (fig. S3). Consistent with this point, the activation-loop mutant K771A increases basal ATPase activity like the C-terminal helix mutations. The loop between the last two helices would act as a hinge that enables a closed-to-open form transition (Fig. 3B). Consequently, the C-terminal tail appears to have a dual role: auto-inhibitory off the membrane and activating on the membrane. Fluorescence resonance energy transfer and lipid sedimentation analyses also show that the C-terminal helix has a role in membrane binding (fig. S6).

The Vps34 ATP-binding pocket (Fig. 4A) has a smaller volume than the corresponding pocket of the class I p110 γ pocket (800 versus 1200 Å³). In Vps34, the P loop [known to bind the phosphates of ATP (26)] curls inward toward the ATP-binding pocket, and this is coincident with a parallel inward bending of the α 11/ α 2

Fig. 1. Structure of Vps34 catalytic core (HELCA). (A) Domain organization of Vps34 and class I PI3Ks. (B) Overall fold of the DmVps34 HELCA. (C) Model for PtdIns head-group binding to Vps34, suggesting that Lys⁸³³-Dm (Lys⁷⁷¹-Hs) interacts with the 1-phosphate (30). (D) View of the hook-shaped activation loop (magenta) encircling the catalytic loop (black). The C2 domain (light blue) is that of p110 γ after superimposing DmVps34 residues 291 to 949 onto p110 γ . The α 12' helix (gray) is the C-terminal helix from the adjacent molecule in the crystal dimer. (E) The putative orientation of Vps34 on a membrane.

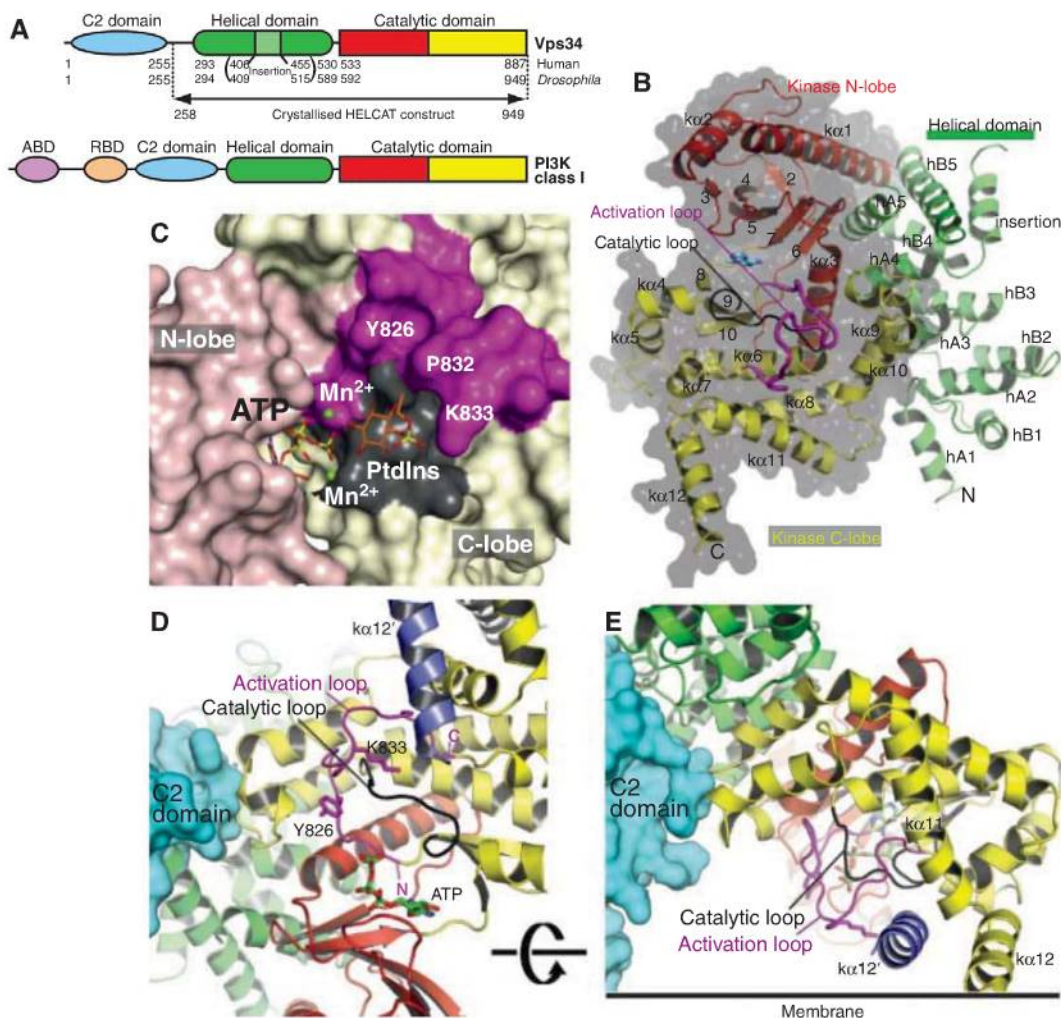


Fig. 2. Essential structural elements for Vps34 catalysis. **(A)** Catalytic activity of human wild-type HELCAT and mutant constructs on PtdIns:PS vesicles. Error bars indicate SD for triplicate assays. **(B)** Proposed catalytic mechanism of Vps34. **(C)** Close-up view of the proposed movements of catalytic loop residues His⁷⁴⁵-Hs and Asp⁷⁴³-Hs between the inactive and active conformations represented by the p110 γ (gray) and DmVps34 (black) crystal structure, respectively. **(D)** The ability of a yeast Vps34p-expressing plasmid to complement the growth defect of a Δ vps34 yeast strain at elevated temperatures is impaired by deletion of the C-terminal helix (Vps34p- Δ C10), a point mutation in this helix (ScH867A), or a mutation in the catalytic loop (ScD731N). **(E)** Basal ATPase activities in the absence of vesicles.

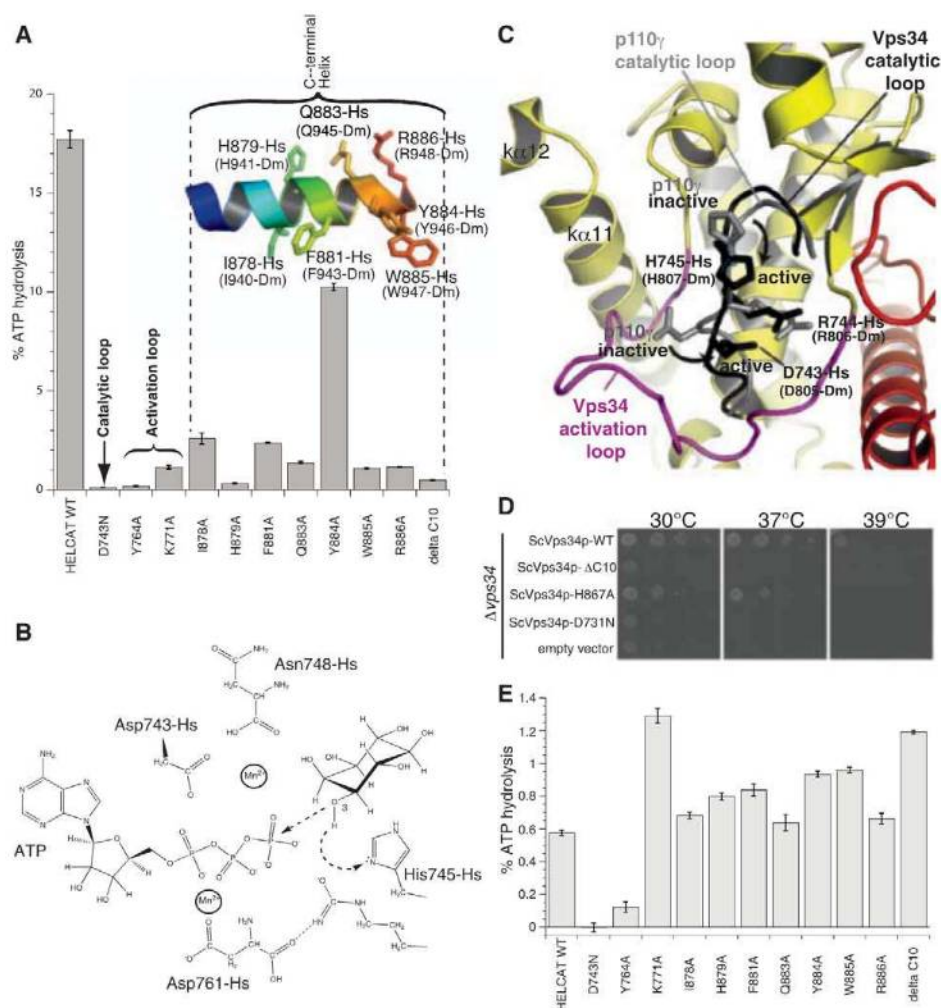
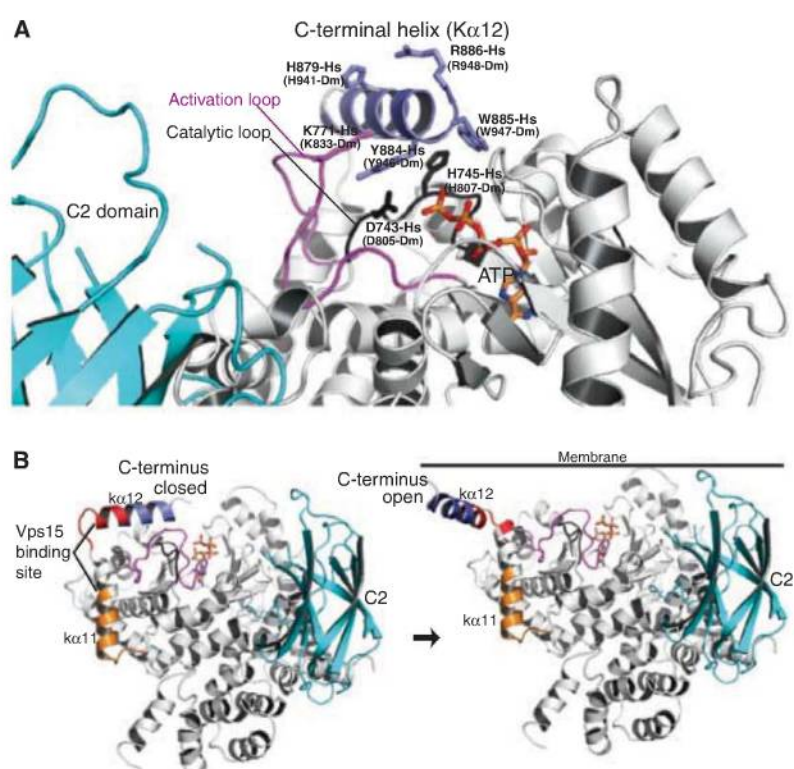


Fig. 3. A model for Vps34 activation on membranes. **(A)** Close-up view for the closed form of the enzyme in the cytosol. The C-terminal helix protects the phosphotransferase center from water. **(B)** The transition of the enzyme from a closed form in the cytosol (C-terminal helix in) to an open form with the C-terminal helix interacting with the membrane. The C2 domain (light blue) is modeled as in Fig. 1. Vps15-interacting regions are classified as strong (red) or weak (orange) (28).



loop (fig. S7). Furthermore, the hinge between the N and C lobes is one residue shorter in Vps34 than in class I PI3Ks; therefore, it lacks the bulged-out space at the adenine-binding pocket hinge, which is characteristic of class I PI3Ks (fig. S8).

Class I PI3Ks can form an allosteric or “specificity” pocket (adjacent to the adenine pocket) only in the presence of propeller-like inhibitors (29). The half-maximal inhibitory concentrations (IC_{50} s) for the propeller-like PI3K inhibitors (e.g., PIK-39) (fig. S9) are generally much worse for Vps34 than other PI3Ks. This is probably due to increased rigidity of the Vps34 pocket arising from a bulky residue substituted in the P loop (Phe⁶¹²-Hs, Phe⁶⁷³-Dm) that packs against the aromatic hinge residue unique to Vps34 (Phe⁶⁸⁴-Hs, Tyr⁷⁴⁶-Dm). These differences effectively close off a corner of the adenine-binding pocket, giving it a more constrained appearance.

Currently there is no high-affinity, specific inhibitor of Vps34. We determined the structure of a complex of Vps34 with 3-methyladenine (3-MA) (Fig. 4, B and C), which is often used as

a specific inhibitor of autophagy. We also determined the structures of Vps34 in complexes with three multi-targeted inhibitors: (i) PIK-90, (ii) PIK-93, (iii) and PI-103 (Fig. 4, D to F). These complexes provide insight into developing more potent and specific Vps34 inhibitors.

Although 3-MA inhibits both class I and III PI3Ks at the 10 mM concentration typically used for inhibiting autophagy in cells, *in vitro* assays show that 3-MA has a preference for Vps34 (fig. S9). There is a hydrophobic ring consisting of Phe⁶⁷³-Dm, Tyr⁷⁴⁶-Dm, and Leu⁸¹²-Dm that encircles the 3-methyl group of 3-MA and is unique to and conserved in Vps34. The corresponding residues in class I PI3Ks are not in close proximity of the 3-methyl group, and the methionine equivalent of Leu⁸¹²-Dm may cause steric hindrance (Fig. 4, B and C). 3-MA appears to bind to the hinge, as does the adenine moiety of ATP in p110 γ , hydrogen bonding to the Val⁷⁴⁷-Dm amide, and the Gln⁷⁴⁵ carbonyl.

All PI3K inhibitors have at least one canonical bond to the hinge. PIK-90 and PI-103 form a single

H-bond, whereas PIK-93 forms two H-bonds to Val⁷⁴⁷-Dm, coinciding with a lower IC_{50} of PIK-93 relative to PIK-90 (fig. S9). The affinity pocket of PI3Ks is lined with several hydrophobic and polar residues with which inhibitors can interact to greatly augment their potency, including Lys⁶⁹⁸-Dm (modified by wortmannin), Asp⁸²³-Dm (from the DFG), and Asp⁷⁰⁶-Dm (in helix α 3, equivalent to helix α C of protein kinases). The pyridine ring of PIK-90, the chlorophenyl group of PIK-93, and the *m*-phenol group of PI-103 are within hydrogen-bonding distance of these residues. In addition, the pyridinylfuranopyrimidine group of PI-103 extends out of the pocket over the surface analogous to hydrophobic region II in protein kinases.

Our initial attempts to synthesize new Vps34 inhibitors, based on the structure of Vps34, indicate that there are ample opportunities to improve their chemical properties and substantially increase specificity for class III PI3Ks. Elaborating the ethanolamine moiety of the PIK-93 sulphonamide (compound 3-94B) that extends out of the affinity pocket and simultaneous elaborations of the sulphonamide and the amide (compound 3-94C) have little impact on IC_{50} values (fig. S10).

To exploit potential differences within the affinity pocket between Vps34 and class I PI3Ks, we increased the steric bulk of the chloro-substituent of the central phenyl ring of PIK-93. Addition of the methoxy group (compound PT21) showed little change in IC_{50} for Vps34 (88 nM), but more than a 10-fold increase in IC_{50} for the most potently inhibited class I PI3K (p110 γ , 61 nM) compared with PIK-93 (fig. S10). To further improve specificity for Vps34, we synthesized an analog of PT21 with additional modifications oriented toward the hinge-region differences between Vps34 and PI3K γ (fig. S8). Compound PT210 (fig. S10) contains a cyclopentanecarboxamide substitution for the acetamide moiety of PIK-93 and exhibits a modest 13-fold loss in potency for Vps34 (IC_{50} = 450 nM) compared with PIK-93, yet PT210 has an 1100-fold higher IC_{50} for PI3K γ (IC_{50} ~ 4 μ M) compared with PIK-93, resulting in a compound with reversed kinase specificity compared with PIK-93.

The structure of Vps34, with a completely ordered phosphoinositide-recognition loop, has enabled us to model substrate binding and the catalytic mechanism. The C-terminal helix plays a critical role in catalysis on membranes. In addition, it also has an auto-inhibitory role that prevents ATP hydrolysis when it is not at the membrane. The structures of Vps34 in complexes with PI3K inhibitors have provided clues as to how 3-MA can preferentially inhibit Vps34, and they have illustrated how additional moieties can be incorporated into inhibitors without affecting affinity for the enzyme, while greatly increasing their specificity for Vps34. This can be crucial in the design of new generations of Vps34 inhibitors with improved specificity, solubility, and cellular availability.

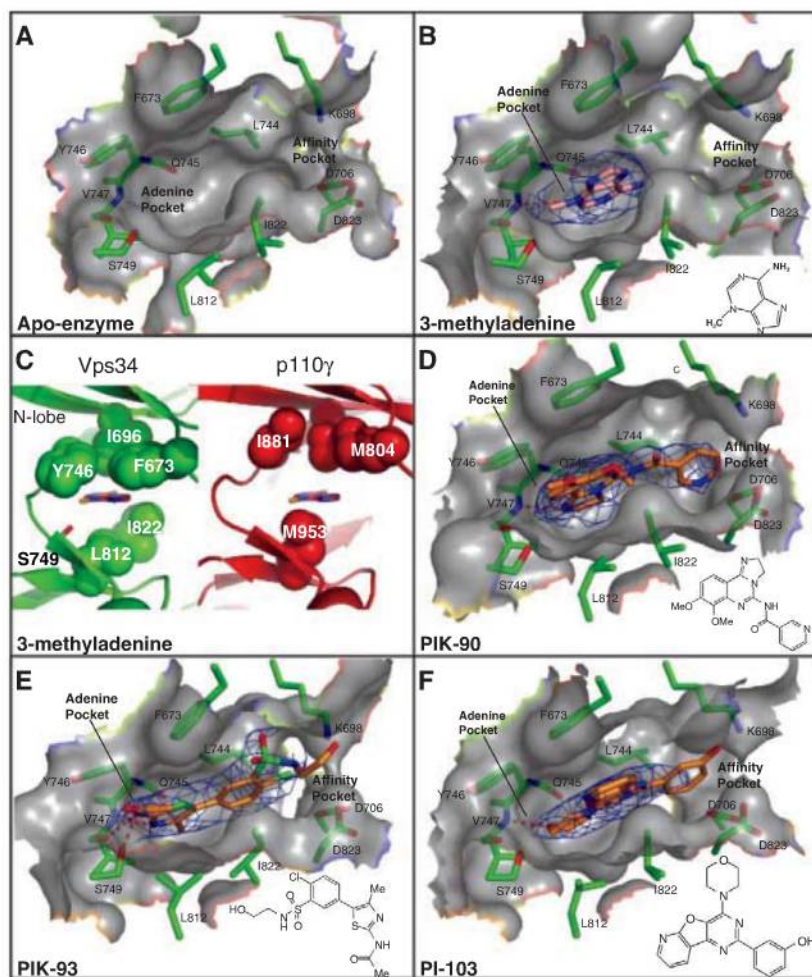


Fig. 4. Inhibitor binding in the ATP pocket. (A) Apo-enzyme. (B, D to F) Inhibitor binding with 2mFo-DFc electron densities shown (contoured at 1.0 σ). (C) A comparison of the ATP-binding pocket of the Vps34/3-MA complex (left, green) with p110 γ (right, red). A ring of hydrophobic residues encircles 3-MA and may provide specificity for Vps34. The p110 γ structure shown is PDB ID 1E8X, but a 3-MA has been placed in the pocket as a reference point for comparison with the Vps34/3-MA complex.

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- Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5973/1638/DC1

Materials and Methods

Figs. S1 to S11

Table S1

References

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Evolutionary Trade-Offs in Plants Mediate the Strength of Trophic Cascades

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Predators determine herbivore and plant biomass via so-called trophic cascades, and the strength of such effects is influenced by ecosystem productivity. To determine whether evolutionary trade-offs among plant traits influence patterns of trophic control, we manipulated predators and soil fertility and measured impacts of a major herbivore (the aphid *Aphis nerii*) on 16 milkweed species (*Asclepias* spp.) in a phylogenetic field experiment. Herbivore density was determined by variation in predation and trade-offs between herbivore resistance and plant growth strategy. Neither herbivore density nor predator effects on herbivores predicted the cascading effects of predators on plant biomass. Instead, cascade strength was strongly and positively associated with milkweed response to soil fertility. Accordingly, contemporary patterns of trophic control are driven by evolutionary convergent trade-offs faced by plants.

Trophic cascades—the indirect positive effect of predators on plant biomass through herbivore suppression—are the best examples of the importance of indirect interactions as determinants of community structure and ecosystem function. For this reason, there has been great interest in elucidating the sources of variation in trophic cascade strength both within (1–3) and among ecosystems (4). Much of the research aimed at explaining variation in trophic cascade strength has focused on factors mediating the top-down effects of predators on herbivores, including the influences of intraguild predation (5), synergistic and antagonistic effects of multiple predators (6), trophic subsidies to predators (7), and the non-consumptive effects of predators on herbivores

(8). At the same time, it is also recognized that plant stoichiometry (9), antiherbivore defense traits (10–12), and primary productivity (13, 14) can mediate trophic cascade strength from the bottom up. Consequently, a consensus is emerging that multiple, complementary top-down and bottom-up processes determine trophic cascade strength.

Although it is recognized that plant traits can influence interactions with herbivores and herbivore-predator interactions (15, 16), there has been little consideration of how plant growth and defense strategies might result in predictable patterns of trophic cascade strength. There is wide acceptance that plant species evolve in response to fundamental trade-offs that should influence the effects of predators and productivity upon herbivore and plant biomass. For example, plant defense theory predicts that fast-growing species should have relatively low herbivore resistance as compared with slow-growing species (17, 18). Plant resistance to herbivores may in turn influence the indirect effects of predators on plants by altering herbivore susceptibility to

predators (19, 20). Similarly, plant growth strategies influence tolerance to herbivory (21, 22), again showing potential to alter the strength of trophic cascades. Although trophic cascades are rightly considered community-level phenomena (23), an understanding of how plant traits influence such dynamics requires first documenting the influence of plant traits on component, species-level cascades.

We conducted a field experiment in which we grew 16 species of milkweeds (*Asclepias* spp., Apocynaceae) (Fig. 1), factorially manipulated predator access and soil fertility, and monitored plant biomass and populations of the potent herbivore *Aphis nerii* (Aphididae, Hemiptera), a specialist on the Apocynaceae that occurs naturally on the studied milkweeds (24). It has previously been shown that milkweed species influence this aphids' population dynamics and interactions with parasitoids (16, 25). We tested whether there are indirect consequences of such variation in trophic dynamics for plant growth as well as whether trade-offs between milkweed growth strategy and herbivore resistance predictably influence the top-down effects of predators. Because all plants were grown in a single environment, any variation in the effects of predators and growth strategy can be attributed to plant species traits. By interpreting these patterns of interspecific variation in trophic structure from a phylogenetic perspective, we link the outcome of fundamental evolutionary trade-offs to contemporary community dynamics.

We first tested for variation among milkweed species in the effects of predators and soil fertility on both plant biomass and herbivore abundance using general linear models. Where species varied in such responses, we then quantified effect sizes for individual species [log response ratios (26)] in order to examine the relationships among species using phylogenetically independent contrasts (27).

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The 16 milkweed species varied in herbivore resistance (quantified here as $-1 \times$ aphid density in the absence of predators) (fig. S1 and table S1) and the top-down effects of predators on herbivore density and plant biomass (Fig. 1 and table S1). This variation in top-down control among species is equivalent in magnitude to that previously observed among ecosystem types (4). That such variation occurs among closely related species in a single abiotic environment, with the same herbivore species and with a common guild of predators, underscores the powerful influence of plant traits upon trophic structure.

Milkweed species also differed strongly with respect to two aspects of the plants' growth strategy, growth rate [which is defined as mean species biomass at the conclusion of the experiment (fig. S1 and table S1)], and the growth response to increased soil fertility (Fig. 1 and table S1). The strength of soil fertility effects on plant biomass was stronger when predators were absent than when present (fig. S2 and table S1), but these dynamics were consistent among milkweed species (table S1). Despite the species-specific effects of soil fertility on milkweed growth, the indirect positive effect of soil fertility on aphid density was indistinguishable among milkweed species (Fig. 1 and table S1). Accordingly, there was asymmetry in how milkweed species influenced top-down and bottom-up trophic dynamics: Predator effects on both herbivore density and plant biomass were species-specific, whereas species variation in soil fertility

effects were limited to the direct influence on plant biomass.

Having shown milkweed species differences in growth strategies, resistance, and the top-down effects of predators, we examined the relationships among these species traits while controlling for phylogenetic history (27). Variation in herbivore density among milkweed species was determined by means of a combination of top-down and bottom-up processes. Although theory and data predict that herbivore resistance in plants should influence predator-herbivore interactions (19, 20), predator effects on herbivores were unrelated to milkweed resistance (fig. S3). In addition, the strength of predator effects on herbivores did not vary as a function of milkweed growth or growth response to soil fertility (table S2). However, both components of milkweed growth strategy convergently traded off against resistance so that species that were fast growing and responsive to soil fertility had low resistance (meaning, higher herbivore densities) (Fig. 2 and table S2), which is consistent with predictions from plant defense theory (17, 18). Thus, herbivore density was jointly and independently determined by means of interspecific variation in the top-down effect of predators and the bottom-up effect of milkweed growth strategies.

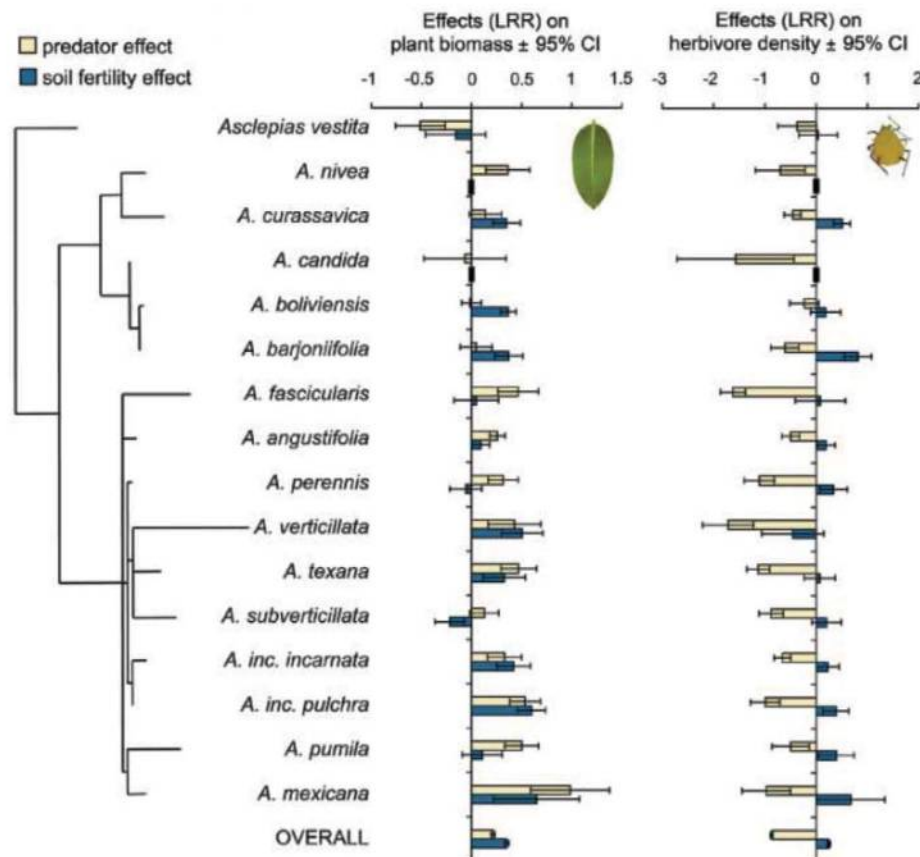
Surprisingly, predator effects on plant biomass were unrelated to either predator effects on herbivores or herbivore density (resistance) (table S2). Instead, plant growth response to soil fertility predicted more than half of the variation

in the top-down effects of predators on plant biomass (Fig. 2). At the same time, milkweed growth rate was not predictive of predator effects on plant biomass [and growth rate and response to soil fertility themselves are uncorrelated (table S2)]. Because the impact of predators on plant biomass was not related to the strength of herbivore suppression, variation in the indirect effects of predators on plants is probably attributable to variation in tolerance of milkweed species to herbivory. Consequently, an evolutionary trade-off leads to an association between high growth in response to soil fertility, low tolerance to herbivory, and an increase in predator effects on plant biomass.

Of several plant traits assayed, we found evidence suggestive of one mechanism behind the observed variation in the top-down effects of predators on plants. Plant emissions of sesquiterpene volatile organic compounds (VOCs) were significantly positively correlated with the top-down effects of predators on plant biomass (fig. S4 and table S3). Sesquiterpenes are a group of VOCs that can play a key role in indirect defense through recruitment of predators to plants (28). Therefore, variation among species in trophic cascade strength may be driven at least in part by interspecific variation in this ecologically important group of volatile compounds.

We have documented wide variation in top-down regulation of plant and herbivore biomass among a group of closely related species; this variation corresponded with a fundamental evo-

Fig. 1. Effect sizes for influence of predators and fertilization on herbivore density (calculated per gram of plant dry biomass) and final plant biomass. Black bars for *A. nivea* and *A. candida* indicate that soil fertility was not manipulated for these species. Effects are natural log response ratios (LRRs) with 95% confidence limits. Predator effects are calculated across both levels of soil fertility, soil fertility effects are calculated across both levels of predation, and all effects are based on manipulation and control sample sizes of $n = 10$ plants each. LRRs of 1.0, 2.0, and 3.0 correspond to changes of 2.7-fold, 7.4-fold, and 20-fold, respectively. Predator effects on both herbivore density and plant biomass differ significantly among species, whereas fertilization effects differ for plant biomass but not for herbivore density (table S1). To the left of the effect sizes, the phylogenetic relationship of the studied milkweeds is presented (29).



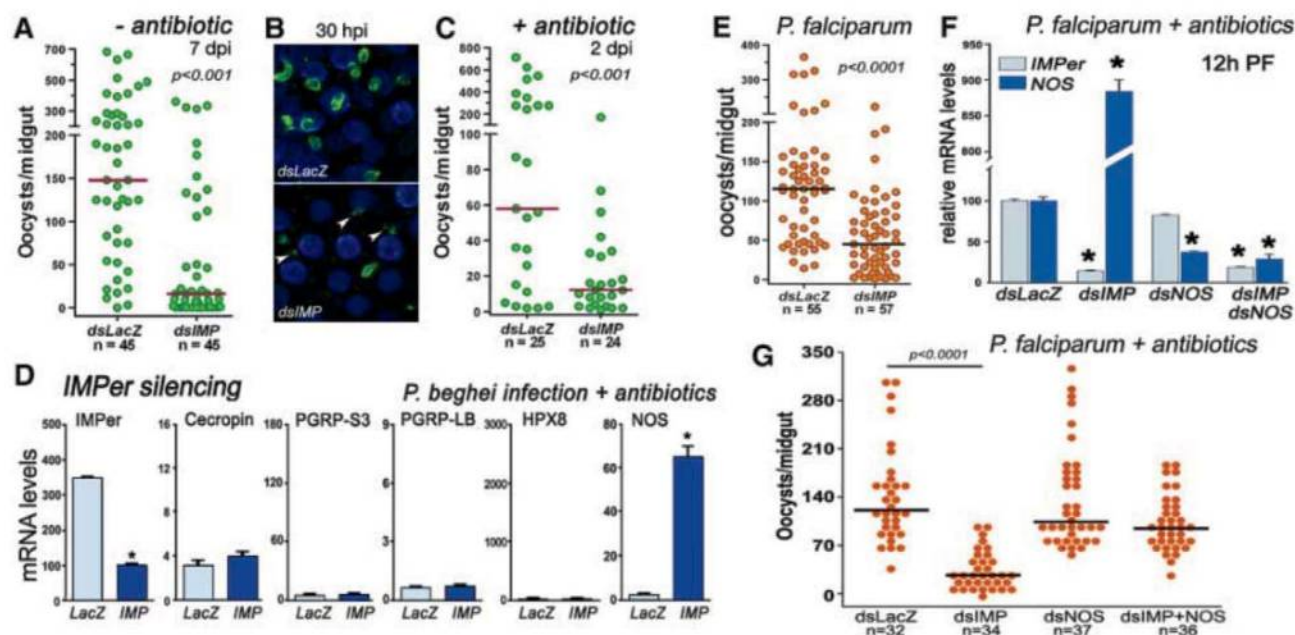
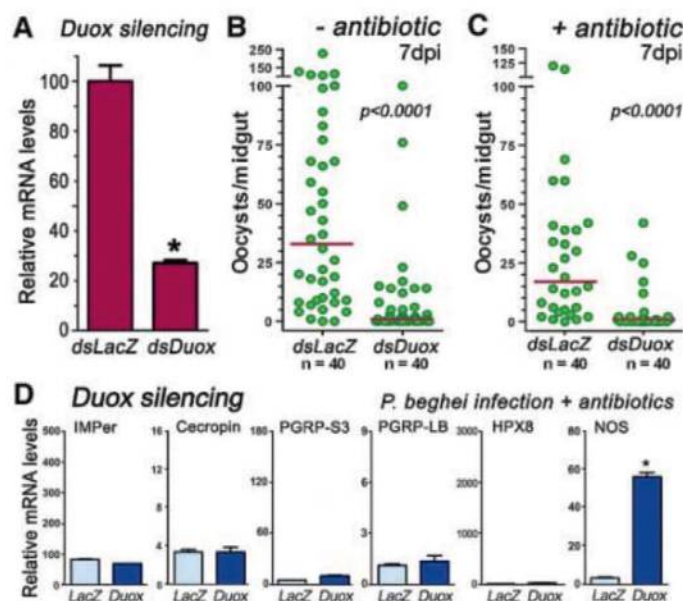


Fig. 2. Effect of IMPer silencing on *Plasmodium* infection and midgut immune activation. Effect of IMPer silencing on *P. berghei* infection (A) 7 dpi, (B) 30 hours post infection (hpi), and (C) 2 dpi in antibiotic-fed mosquitoes. Arrowheads in (B) indicate fragmented parasites. (D) IMPer, cecropin, PGRP-S3, PGRP-LB, HPX8, and NOS mRNA levels in control (dsLacZ-injected) and IMPer-silenced midguts of antibiotic-treated mosquitoes at 24 hpi (mean $\pm$ SEM). (E) Effect of IMPer silencing on *P. falciparum* in-

fection at 8 dpi. (F) Effect of silencing IMPer or NOS (or co-silencing IMPer and NOS) on IMPer and NOS mRNA expression 12 hpi in antibiotic-fed mosquitoes infected with *P. falciparum* (mean $\pm$ SEM) and (G) *P. falciparum* infection at 7 dpi. Asterisks indicate significant differences relative to the dsLacZ control. Each circle represents the number of parasites in an individual midgut, and the horizontal lines indicate the medians.

dues (dityrosine bonds) (11, 12). Our initial findings suggest that IMPer could be required to form a barrier that limits the rate of diffusion of immune elicitors and prevents immune activation. Given the negative effect of IMPer silencing on bacterial growth, we next asked whether IMPer also affects *Plasmodium berghei* (rodent malaria) survival. IMPer silencing reduces the median number of *P. berghei* oocysts present 7 days post infection (dpi) by 9.2-fold (Fig. 2A). This effect is already observed early in the invasion process (30 hours after feeding), when the number of intact ookinetes is greatly reduced (fig. S5). In IMPer-silenced females, ookinetes invade the midgut but are killed and appear fragmented (Fig. 2B). The drastic reduction of *Plasmodium* infection in IMPer-silenced mosquitoes is not due to activation of antibacterial responses, as it is also observed in females pretreated with oral antibiotics (Fig. 2C). As expected, antibacterial markers are not induced when antibiotic-treated females are infected with *Plasmodium*, indicating that IMPer silencing activates these genes only when bacterial elicitors are present in the midgut lumen. Instead, there is a dramatic induction of NOS, an enzyme that generates nitric oxide (a potent antiparasitic effector molecule) (Fig. 2D). These findings indicate that IMPer is required for *Plasmodium* parasites to develop in the midgut without activating the immune pathway(s) that regulate NOS expression. We have previously shown that overactivation of the signal

Fig. 3. Effect of Duox silencing on *Plasmodium* infection and midgut immune activation. (A) Midgut Duox silencing at 4 days after injection of dsDuox. (B) Effect of Duox silencing on *P. berghei* infection at 7 dpi. (C) Same as in (B), but in antibiotic-fed females. (D) Effect of Duox silencing on cecropin, PGRP-S3, PGRP-LB, hemeperoxidase 8, and NOS expression in midguts of antibiotic-treated mosquitoes collected at 24 hpi (mean $\pm$ SEM).



transducers and activators of transcription pathway induces high levels of NOS at 4 dpi that greatly reduce oocyst survival (13), but the abnormal induction of NOS in IMPer-silenced mosquitoes is observed earlier.

Plasmodium falciparum infection is also greatly reduced in IMPer-silenced *A. gambiae* (Fig. 2, E and G) and *A. stephensi* (fig. S6) at 7 dpi. Furthermore, IMPer silencing in antibiotic-treated

A. gambiae mosquitoes infected with *P. falciparum* also triggers a strong induction of midgut NOS expression 12 hours after feeding (Fig. 2F) and reduces infection ($P < 0.0001$) (Fig. 2G). At this time, immature ookinetes are still developing in the blood bolus within the PM matrix. When IMPer is not present, epithelial cells can detect *Plasmodium* immune elicitors, possibly glycosylphosphatidylinositols (GPIs), which activate

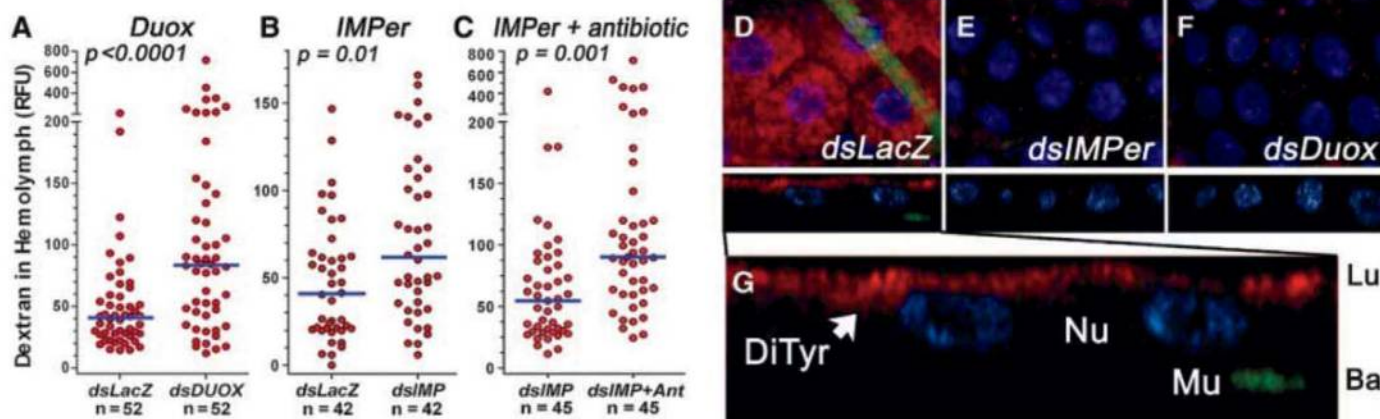


Fig. 4. Gut permeability and dityrosine network. Effect of (A) Duox or (B) IMPer silencing without or (C) with oral antibiotics (Ant) on midgut permeability to fluorescent dextran (4 kD). Each circle represents fluorescence in the hemolymph of an individual mosquito 18 to 20 hours after feeding. Horizontal lines indicate the medians. RFU, relative fluorescence units. (D

to G) Immunofluorescence staining of midguts 14 hours after feeding. Dityrosine bonds (red) and muscle actin (green) in mosquitoes injected with (D) dsLacZ, (E) dsIMPer, or (F) dsDUOX. (G) Enlargement of image in (D). DiTyr, dityrosine staining; Lu, lumen; Ba, basal; Mu, muscle; and Nu, nuclei.

NOS expression. Previous studies have shown that oral administration of *Plasmodium* GPIs activates midgut NOS expression through the Akt/protein kinase B pathway (14). To determine whether high levels of NOS mediate parasite killing, we silenced both IMPer and NOS. NOS mRNA levels are 30-fold less in the double-silenced mosquitoes than when only IMPer is silenced (Fig. 2F). Lower NOS levels prevent the deleterious effect of IMPer silencing on *Plasmodium* infection (Fig. 2G), indicating that the antiparasitic effect of IMPer silencing is mediated by NOS, probably by increasing the rate of nitration when parasites invade gut epithelial cells (15).

Duox generates hydrogen peroxide, a substrate required for IMPer to be active, on the luminal surface of epithelial cells. We investigated whether Duox is also required to prevent activation of antiparasitic responses. dsDuox silencing reduces midgut Duox mRNA levels by 77% (Fig. 3A) and drastically reduces *Plasmodium* infection in the presence (Fig. 3B) or absence (Fig. 3C) of bacteria. This phenotype is very similar to that observed when IMPer is silenced (Fig. 2A). In antibiotic-treated females infected with *Plasmodium*, Duox silencing does not induce expression of the antibacterial markers (Fig. 3E); however, NOS expression is highly induced. Together, our data support the hypothesis that IMPer and Duox are both required to prevent activation of midgut responses to *Plasmodium* immune elicitors.

We propose a model (fig. S7) in which the IMPer/Duox system mediates protein cross-linking by forming dityrosine bonds. This network of covalently linked proteins decreases the rate of diffusion of immune elicitors, decreasing their interaction with pathogen recognition receptors on the surface of midgut cells. In agreement with this model, silencing Duox (Fig. 4A) or IMPer (Fig. 4B) or reducing immune elicitors with oral antibiotics (fig. S8) significantly increases the rate

of absorption of a fluorescent dextran administered in the blood meal, indicating that the dextran has increased access to the gut surface. Decreasing immune elicitors in IMPer-silenced mosquitoes further enhances gut permeability (Fig. 4C). The dityrosine network is dynamic and probably transient, as IMPer is expressed 6 to 18 hours after feeding, a time when bacteria proliferate but blood digestion is not fully active.

We used monoclonal antibodies to detect dityrosine bonds on the midgut surface. A network of dityrosine-linked proteins is observed on the luminal surface of epithelial cells of blood-fed control mosquitoes injected with dsLacZ (Fig. 4, D and G) but is absent when either IMPer (Fig. 4E) or Duox (Fig. 4F) is silenced, in agreement with the proposed model.

In conclusion, *A. gambiae*, midgut epithelial cells have the ability to activate pathogen-specific responses to bacteria and *Plasmodium* and to modulate the permeability of the mucus layer to soluble molecules present in the blood bolus. The dityrosine network formed by the IMPer/Duox system allows bacteria to proliferate without activating epithelial immunity but also makes mosquitoes more susceptible to *Plasmodium* infection, as parasites can develop within the midgut lumen without being detected. In *Drosophila*, silencing of a secreted peroxidase results in high mortality when flies are fed live or dead bacteria, but not when they are fed sterile food. Furthermore, antioxidants can rescue this mortality, indicating that high levels of reactive oxygen species (ROS) are mediating death (16). Our studies suggest that this enzyme may also be involved in the formation of a dityrosine network in *Drosophila* and that disruption of this barrier could result in chronic immune activation and ROS generation. In mosquitoes, IMPer or Duox silencing does not affect survival (fig. S9), probably because mosquitoes are batch feeders, and gut bacteria and blood-meal remnants are expelled 2 to 3 days after feeding.

The Duox system appears to use a dynamic two-prong strategy to protect epithelial cells from potential pathogens. Bacterial elicitors are known to activate Duox activity quickly through the phospholipase C β signaling pathway (3, 17). This would generate hydrogen peroxide and activate IMPer, forming the dityrosine network and decreasing the permeability of the mucus layer to immune elicitors. Expression of microbicidal effector genes would be induced when this initial response cannot prevent contact of immune elicitors with pathogen recognition receptors on the surface of epithelial cells; for example, when pathogenic bacteria or *Plasmodium* parasites breach the PM and the mucus barriers. These two complementary mechanisms would allow an effective immune response while minimizing the deleterious effects that chronic activation of potent effector molecules could have on commensal bacteria and on the host.

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Supporting Online Material

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A Self-Incompatibility System Explains High Male Frequencies in an Androdioecious Plant

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Androdioecy is a sexual system in which males co-occur with hermaphrodites, which have both male and female function. Stable androdioecy is rare in nature, and theory suggests that it requires that males sire more than twice as many offspring as hermaphrodites. In several members of the olive family (Oleaceae), androdioecy occurs with higher frequencies of males than predicted by theory. In *Phillyrea angustifolia* L., we found that high male frequencies can be maintained in natural populations because hermaphrodites belong exclusively to one of two self-incompatibility groups, and thus, each can fertilize only half of all pollen recipients. In contrast, males can pollinate all hermaphrodites. Thus, in this species, the reproductive disadvantage that males face due to the loss of female function is offset by the fact that all males are fully compatible with all pollen recipients.

Most plant species are hermaphroditic, and individuals have both male and female function. However, populations of hermaphrodites containing individuals with only male or female function have evolved independently in numerous lineages. These populations may represent an intermediate stage between a fully hermaphroditic mating system and dioecy, a mating system where all individuals have only male or female function (1). The most frequently reported intermediate stage in plants is gynodioecy, where females invade a hermaphroditic population via a male-sterility mutation, and the remaining hermaphrodites then evolve to become progressively more male (2). Dioecy also may evolve via female sterility,

resulting in an androdioecious population (1). However, theory predicts that the evolution of gynodioecy or androdioecy from hermaphroditism should be difficult because, unless accompanied by a means to double their fitness, male- or female-sterile individuals will have half the reproductive capability of hermaphrodites, which can utilize both pollen and ovules for reproduction (1, 2).

In plants, gynodioecy is relatively common (3); its success is explained by nucleocytoplasmic conflict (4). Male sterility is encoded by mitochondrial variants that are maternally transmitted (5) and, therefore, do not suffer from the reproductive disadvantage linked to the loss of male function; in some cases, they may even benefit from a reproductive advantage if male resources are reallocated to female function (6–9). Moreover, selfing associated with inbreeding depression can make it easier for gynodioecy to evolve. In contrast, high frequencies of males in androdioecious populations cannot be explained, as most female sterility appears to be Mendelian. According to theory, to be maintained in the population, males have to sire more than twice as many offspring as the hermaphrodites with which they co-occur (10). An even greater advantage is required in partially self-fertilizing populations, because fewer ovules in hermaphrodite recipients will be available for outcrossing (1, 10–13). Thus, a functionally androdioecious species is expected to exhibit low male

frequencies in populations, self-incompatibility (SI) or at least a low selfing rate, and a large siring advantage for males.

Several species that have been described as morphologically androdioecious show a mating system more akin to a dioecious species in which hermaphrodites do not have an efficient male function (10). Only four animal genera and eight plant species have actually been confirmed as androdioecious (14, 15). As in other Oleaceae species (16, 17), the mating system of *Phillyrea angustifolia* is unclear (18) because this species has female-sterile individuals at frequencies that can reach or even exceed 50% in natural populations (19). In theory, such high frequencies require (1, 11) an infinite male advantage and that hermaphrodites reproduce via their female function only. Accordingly, it has been suggested that the high frequency of males encountered in *P. angustifolia* populations may actually indicate functional dioecy (14).

Controlled crosses (20) in open-field conditions (21) have not provided support for this theory: They demonstrate that hermaphrodites effectively have male function and that males enjoy only a low, insignificant fitness advantage (20, 21), which cannot explain their high proportions. We performed controlled crosses in natural populations with hermaphrodites sired by pollen either from males or from hermaphrodites (22) (table S1) and verified the crosses using paternity analysis with polymorphic microsatellite markers (21, 23). These crosses revealed that female sterility is dominant and transmitted exclusively by males. Using additional controlled crosses, we determined incompatibility phenotypes with a stigma test, where we scored the presence or absence of pollen tubes in the stigma using fluorescent microscopy (20) (Fig. 1). Testing 107 hermaphrodites from five Mediterranean populations separated by 10 to 1000 km (fig. S1), we identified two groups of self-incompatible hermaphrodites (G1 and G2): In each group, plants were incompatible with each other but compatible with the members of the other group (Fig. 1 and Table 1) with no exceptions (table S2).

In contrast, all crosses performed between males and G1 and G2 hermaphrodite recipients were compatible (table S3) (20). Cross-compatibility assessed by the stigma test was subsequently confirmed by fruit production (table S3). Thus, males exhibit consistent and exclusive compatibility with all hermaphrodites tested so far, indicating that sex-determining

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and incompatibility loci are genetically linked. Hence, even when males and hermaphrodites produce the same amount of pollen, males can potentially sire twice as many ovules as hermaphrodites. This fitness advantage may be even higher if males are able to reallocate their resources into higher pollen production and/or flower production (24).

The fact that only two incompatibility groups were detected in hermaphrodites ruled out the possibility of gametophytic genetic control of self-incompatibility (GSI) in *P. angustifolia*. In GSI, the incompatibility (denoted S) gene is expressed in the haploid pollen grain after meiosis; this incompatibility system requires a minimum of three alleles and, consequently, a minimum of three incompatibility types to be functional (25). Alternatively, in sporophytic genetic control of self-incompatibility (SSI), the S gene is expressed before meiosis in the diploid sporophytic tissue, and incompatibility can arise with only two alleles, with a complete dominance of one allele over the other. Therefore, we hypothesize that *P. angustifolia* has an SSI governed by an S-locus with two alleles, S2 and S1 (with S2 dominant over S1), producing the two incompatibility groups G1 and G2. We predict that one group represents the recessive homozygote S1S1, and the other the S1S2 heterozygote.

Diallelic incompatibility in a homomorphic, wind-pollinated species has never before been observed in a homostylous plant genus (where styles—the pollen-conducting tube between the stigma and the ovule of a flower—are the same length in all individuals), although it has been shown to occur in the case of distyly (where styles vary in length, generally linked to incompatibility loci). In theory, diallelic incompatibility evolves either in heterostylous plants (26) or from a homostylous self-compatible plant (27), which suggests the existence of—at least transient—diallelic incompatibility in a homomorphic species.

Phylogenetic analysis suggests that the ancestral status of Oleaceae is diploid and distylous (28). Derived allopolyploid taxa, including the Oleinae subtribe to which *P. angustifolia* belongs, underwent hybridization and genome doubling and are mainly homostylous (29). Hence, during the breakdown of heterostyly, which may have occurred through allopolyploidization or in relation to a change in pollination system (30), the diallelic incompatibility system appears to have been conserved. The strict association between male phenotype and the full compatibility with G1 and G2 hermaphrodites requires genetic linkage between a gene determining sexual development (e.g., inhibiting stigma development) and either the self-incompatibility locus itself (31) or a modifier of self-incompatibility located elsewhere in the genome. Lending support to this theory, the self-incompatibility gene has a dual role in self-incompatibility and pistil development (32).

Whatever its origin, the occurrence of diallelic incompatibility in a wind-pollinated species has generated conditions that have driven evolution toward an androdioecious mating system, with the appearance of and maintenance of male individuals at higher-than-predicted frequencies within populations. Nevertheless, the

maintenance of a diallelic incompatibility system in hermaphrodites, which is not associated with morphological differences, is surprising because these systems are susceptible to rapid invasion by new incompatibility alleles, which will experience a strong negative frequency-dependent advantage. This is true even after androdioecy

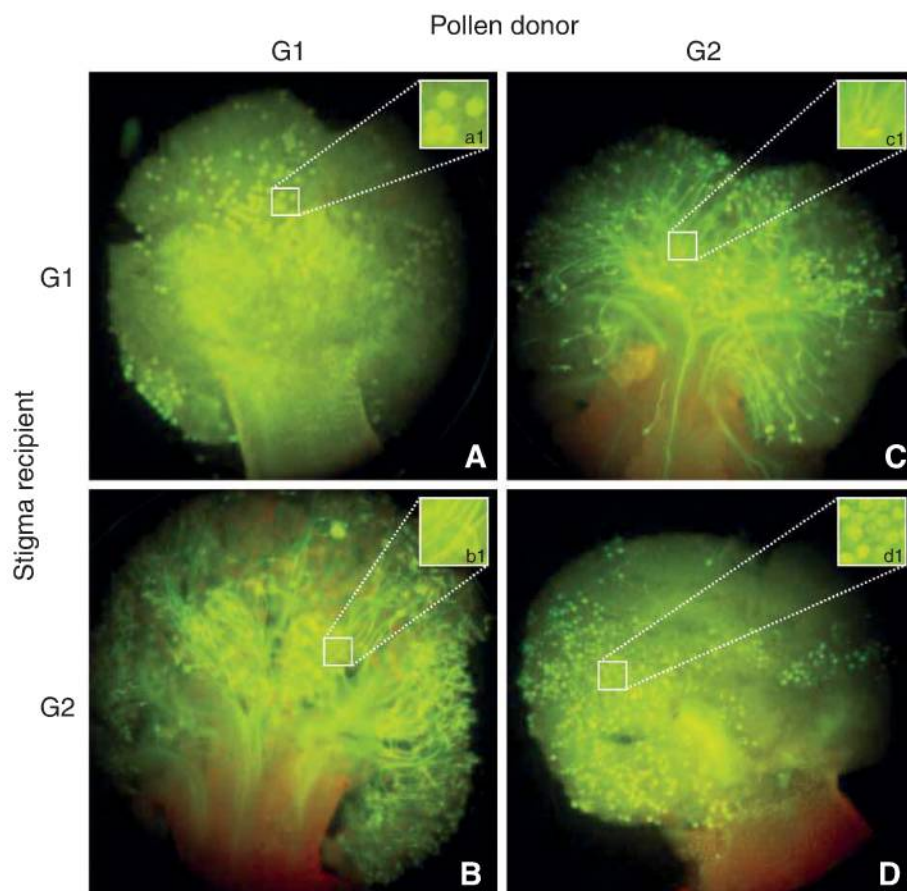


Fig. 1. Stigma tests. Stigmas of hermaphrodite recipients belonging to G1 are incompatible with pollen from G1 donors (A) but compatible with pollen from G2 donors (C). A strict symmetry is observed on stigmas of recipients belonging to G2: compatibility when pollinated by G1 (B), incompatibility when pollinated by G2 (D). (Insets) a1 and d1: zoom on nongerminated pollen grains; b1 and c1: zoom on pollen tubes.

Table 1. Two incompatibility groups detected among hermaphrodites sampled on a wide geographic scale: results of the stigma test. For numbered study sites, see map in fig. S1. Incompatibility groups in hermaphrodites are called G1 and G2; G1, hermaphrodites incompatible with each other and compatible with plants of the G2 group; G2, hermaphrodites incompatible with each other and compatible with plants of the G1 group. Of the 107 crosses performed, no cases of dual compatibility with both G1 and G2 were detected.

Study site	Number of hermaphrodite pollen recipients compatible with reference pollen donors		
	G1	G2	G1 and G2
1. Fabrègues	16	16	0/32
2. Moissac-Vallée-Française	10	9	0/19
3. Île Sainte-Lucie	9	10	0/19
4. Camargue—La Tour du Valat	16	2	0/18
5. Cadiz-Pinar de la Algaida	14	5	0/19
Total number of tested individuals	65	42	0/107

is established, because androdioecy itself does not favor the maintenance of only two incompatibility groups among hermaphrodites within populations. Investigations are now needed to determine the function and mechanism maintaining the low number of S alleles in *P. angustifolia* and any possible constraints that prevent the appearance of new incompatibility alleles.

Up to now, the high proportions of males in populations, sex determination, and relations among sexual phenotypes were inexplicable. This study at last provides an explanation by demonstrating the existence of unusual incompatibility relations among hermaphrodites. This incompatibility system may be more widespread, not only driving the diversification of the Oleaceae mating systems, but also other cases of distyly. This work supports previous hypotheses and models regarding the invasion of males in hermaphroditic populations (10, 25, 33). Until now, these theoretical ideas were largely contradicted by empirical examples of functional androdioecy that evolved from dioecious mating systems (3, 14, 34). Our results provide an alternative transition from hermaphroditism to androdioecy.

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Supporting Online Material

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Materials and Methods

Fig. S1

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The Wnt/ β -Catenin Pathway Is Required for the Development of Leukemia Stem Cells in AML

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Leukemia stem cells (LSCs) are capable of limitless self-renewal and are responsible for the maintenance of leukemia. Because selective eradication of LSCs could offer substantial therapeutic benefit, there is interest in identifying the signaling pathways that control their development. We studied LSCs in mouse models of acute myelogenous leukemia (AML) induced either by coexpression of the *Hoxa9* and *Meis1a* oncogenes or by the fusion oncoprotein MLL-AF9. We show that the Wnt/ β -catenin signaling pathway is required for self-renewal of LSCs that are derived from either hematopoietic stem cells (HSC) or more differentiated granulocyte-macrophage progenitors (GMP). Because the Wnt/ β -catenin pathway is normally active in HSCs but not in GMP, these results suggest that reactivation of β -catenin signaling is required for the transformation of progenitor cells by certain oncogenes. β -catenin is not absolutely required for self-renewal of adult HSCs; thus, targeting the Wnt/ β -catenin pathway may represent a new therapeutic opportunity in AML.

Acute myelogenous leukemia (AML) is the most common acute leukemia in adults, and most patients are not cured with current therapies. Only a small subset of AML cells are capable of extensive proliferation and self-renewal (1). Such cells are referred to as leukemia stem cells (LSCs) because they

share properties, including extensive self-renewal potential, with normal stem cells. LSCs are being studied as potential therapeutic targets (2, 3), and the signaling pathways that control their development and survival are of particular interest. The Wnt/ β -catenin pathway is active in certain human leukemias (4–7) and in normal

hematopoietic stem cells (HSCs) (8–10). Although β -catenin is not required for self-renewal of adult HSC (11, 12), it is unclear whether β -catenin is required for LSC development and maintenance in AML. We have studied well-characterized mouse models of AML to explore whether β -catenin is necessary for LSC development from either HSC or more differentiated committed myeloid progenitor cells.

Homeobox (Hox) genes have been implicated in the regulation of normal stem cell self-renewal (13, 14), and enforced coexpression of *Hoxa9* and *Meis1a* in mouse bone marrow leads to rapid AML development (15). Previous studies demonstrated that leukemogenic fusion proteins, such as those involving the mixed lineage leukemia (MLL) protein (for example, MLL-AF9), can transform non-self-renewing granulocyte/macrophage progenitors (GMP) and activate

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genes encoding Hox proteins such as HoxA9 and Meis1 (16–18). To determine whether a combination of Hoxa9 and Meis1a (HoxA9/M) can transform GMP, we cotransduced sorted Lin[−] c-Kit⁺ Sca-1⁺ (KLS) cells, which are enriched for HSC, or Lin[−] c-Kit⁺ Sca-1[−] Fcγ⁺ CD34⁺ (GMP) cells with viruses encoding HoxA9 (MSCV-HoxA9-GFP) and Meis1a (MSCV-Meis1a-puro), and used either a bulk approach (15, 18, 19) or a single-cell approach (fig. S1). When single cells were expanded in vitro up to 6 weeks, no differences were observed between KLS cells transduced with HoxA9/M (KLS-HoxA9/M), GMP transduced with HoxA9/M (GMP-HoxA9/M), or GMP transduced with MLL-AF9 (GMP-MLLAF9) in proliferation, cell-cycle profile, apoptosis levels, gross morphology, or immunophenotype (fig. S1). However, there was a proliferative defect upon extended culture of GMP-HoxA9/M cells. When cells were injected into recipient mice, 13 of 14 (93%) mice that received GMP-MLLAF9 cells developed AML and 14 of 20 (70%) mice that received KLS-HoxA9/M cells developed AML, whereas only 1 of 23 mice that received GMP-

HoxA9/M cells developed AML (fig. S2). These differences in leukemogenic potential could not be ascribed to differential expression of *HoxA9* and *Meis1* (fig. S3).

To characterize the in vivo defect in HoxA9/M-transduced GMP, we monitored green fluorescent protein-positive (GFP⁺) cells and their immunophenotypes in recipient mice at multiple timepoints after transplantation. By 18 hours, KLS-HoxA9/M and GMP-HoxA9/M cells homed to the bone marrow with similar efficiency (fig. S2). By 8 days, both cell types displayed heterogeneity of expression of the stem cell marker, c-Kit (c-Kit^{high} and c-Kit^{low}) (Fig. 1A). KLS-HoxA9/M cells sustained the two c-Kit populations through 1 month, until ultimately a lethal leukemia developed (Fig. 1A). Conversely, at 1 month after transplantation, GMP-HoxA9/M retained only the c-Kit^{low} population that was eventually lost from bone marrow (Fig. 1A).

To determine whether the c-Kit^{high} and c-Kit^{low} populations that were identified 1 month after injection of KLS-HoxA9/M cells are func-

tionally distinct, we used in vivo limiting dilution analysis to measure the frequency of leukemia-initiating cells (LICs) in these two populations. LICs are defined as cells isolated before the development of clinically evident leukemia that can initiate leukemia in subsequent recipient mice. A high frequency of LIC was observed in the c-Kit^{high} population. In contrast, c-Kit^{low} cells were inefficient at inducing leukemia (fig. S4). We next assessed LSC activity in the fully developed HoxA9/M-induced leukemias. Limiting dilution analysis demonstrated that the Gr1^{−/low} c-Kit^{high} population was over 100-fold enriched for LSC as compared with that of the Gr1⁺ c-Kit^{high} cells (figs. S4 and S5) and had an immunophenotype similar to MLL-AF9 LSC (L-GMP) (18, 20) (fig. S6). Thus, maintenance of the c-Kit^{high} population is associated with leukemia development, and cellular heterogeneity is found throughout the process of leukemia development. The inability of GMP-HoxA9/M cells to induce leukemia prompted us to search for a self-renewal pathway active in LSC that might be deficient in the GMP-HoxA9/M cells.

We previously demonstrated that Lin[−] Sca-1[−] c-Kit^{high} Fcγ⁺ CD34⁺ cells (termed L-GMP) are enriched for LSC in the MLL-AF9 AML model and that L-GMP are globally similar to normal cells at a mid-myeloid stage of development but express a subset of genes normally highly expressed in HSC (18). Given that the LSC population in HoxA9/M-driven leukemia described here has a similar immunophenotype, we re-

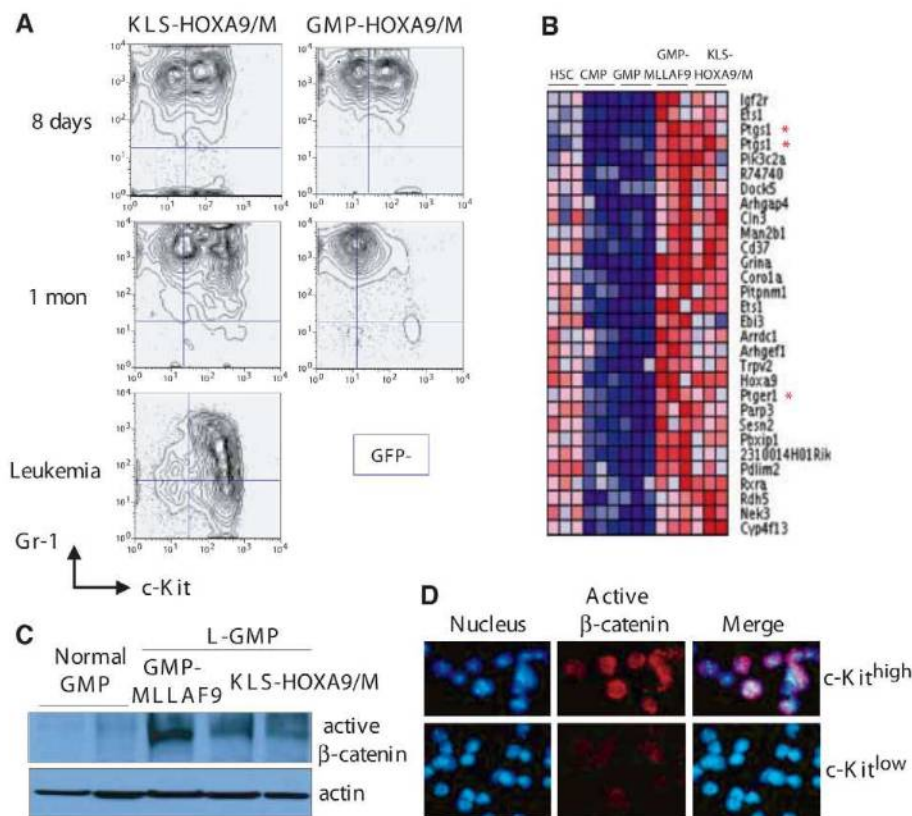


Fig. 1. β-catenin is activated in developing leukemia cells and L-GMP derived from KLS-HoxA9/M and GMP-MLLAF9. (A) Immunophenotypic analyses of GFP⁺ cells from bone marrow of mice transplanted with pre-leukemia KLS-HoxA9/M or GMP-HoxA9/M cells at 8 days, 1 month, or 4 months (leukemia) after transplantation. (B) The heat map displays the top 30 probe sets for genes that show increased expression in the HSC population (KLS cells) and L-GMP by use of comparative marker selection and permutation testing (200 probe sets passed a cutoff of $P < 0.002$). Expression of *Ptgs-1* (Cox-1) and *Ptger1* (prostaglandin E receptor 1) is shown. (C) Immunoblot analysis for active (dephosphorylated) β-catenin in normal GMP or L-GMP derived from GMP-MLLAF9 or KLS-HoxA9/M mediated leukemias. (D) Immunofluorescence assessment for active β-catenin in c-Kit^{high} or c-Kit^{low} populations isolated from mice 1 month after injection of pre-leukemia KLS-HoxA9/M cells. Red, active β-catenin; blue, Hoechst; merge, active β-catenin/Hoechst.

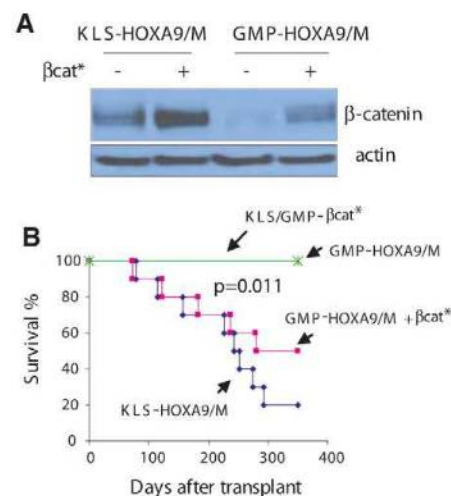


Fig. 2. Constitutively active β-catenin cooperates with HoxA9/M to induce AML from GMP cells. (A) Constitutively active βcat* was transduced into pre-leukemia KLS-HoxA9/M or GMP-HoxA9/M cells, and protein levels were assessed by means of immunoblot analysis. (B) Survival curves of mice receiving KLS or GMP cells transduced with active βcat* (controls), GMP-HoxA9/M, GMP-HoxA9/M cells transduced with active βcat*, or KLS-HoxA9/M. 5×10^5 cells were transplanted into sublethally irradiated recipients ($n = 10$ recipients in each group). P was determined by using the log-rank test.

soned that genes highly expressed in MLL-AF9 L-GMP, HoxA9/M L-GMP, and HSC but not in GMP might pinpoint genes that are important for leukemic self-renewal. Supervised analysis of gene expression data demonstrated a group of genes that are highly expressed in normal and leukemia stem cells and expressed at lower levels in normal myeloid progenitors (Fig. 1B). Prostaglandin-endoperoxide synthase 1 (Ptgs1) (also known as Cyclooxygenase-1 or Cox-1) and one of the pros-

taglandin receptors (Ptger1) were up-regulated in both GMP-MLLAF9-derived and KLS-HoxA9/M-derived L-GMP, which was confirmed through quantitative polymerase chain reaction (Fig. 1B and fig. S6). Because previous studies have highlighted a critical connection between prostaglandin synthesis and the Wnt/ β -catenin pathway (21–24), we measured β -catenin activation. An antibody specific for activated β -catenin demonstrated activity in GMP-MLLAF9-derived and KLS-HoxA9/M-

derived L-GMPs but not in normal GMP, as assessed by means of immunoblot or immunofluorescence (Fig. 1C and fig. S7). Also, β -catenin activity was found in GMP-MLLAF9-derived and KLS-HoxA9/M-derived cells grown in vitro but not in GMP-HoxA9/M-derived cells (fig. S7). Activated/nuclear β -catenin is found in c-Kit^{high} but not c-Kit^{low} cells isolated from mice 1 month after injection with KLS-HoxA9/M cells (Fig. 1D).

Fig. 3. Pharmacological inhibition of β -catenin impairs LSC function. (A) Immunoblot analysis of β -catenin levels in c-Kit^{high} cells sorted from bone marrow at 1 month after injection of mice with KLS-HoxA9/M cells, which were subsequently grown in methylcellulose with and without exposure to indomethacin for 3 weeks (19, 26). (B) Immunophenotypic analysis of bone marrow GFP⁺ cells after injection of KLS-HoxA9/M cells and treatment with either vehicle or indomethacin (repeated with 2 independent clones) for 7 days. Treatment began at day 3 after injection, and mice were sacrificed at day 10 after transplantation (19, 23). (C and D) Survival curves of mice (five mice for each group) injected with the indicated number (10 to 10^4) of GFP⁺ marrow cells sorted from control or Indo-treated mice that had received pre-leukemia KLS-HoxA9/M cells (C) or leukemic GMP-MLLAF9 cells (D) (repeated with two independent clones).

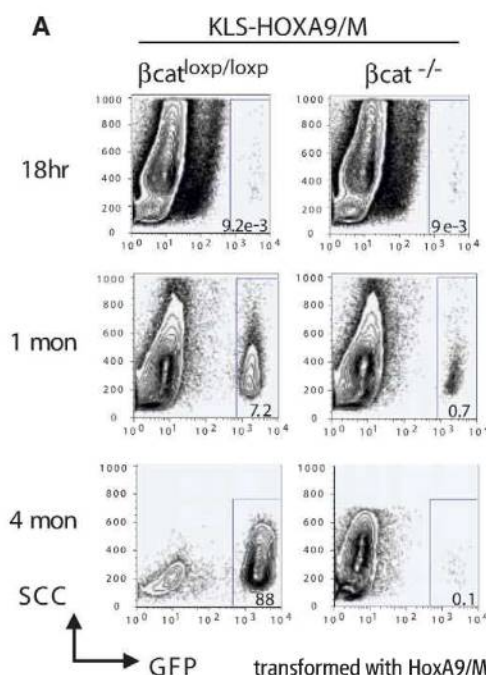
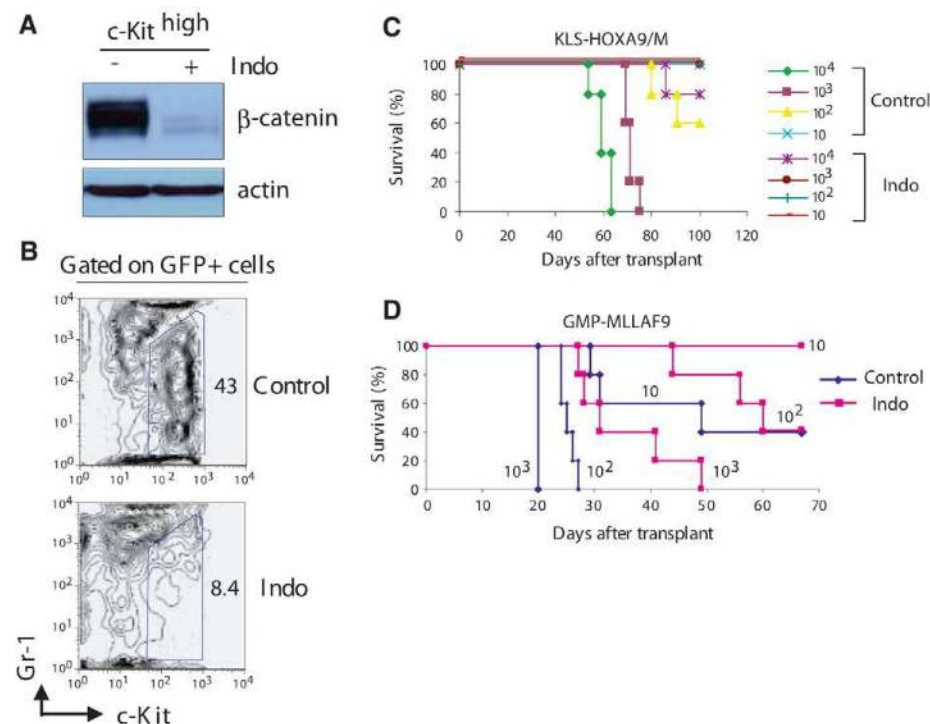


Fig. 4. Deletion of β -catenin impairs the development of AML induced by KLS-HoxA9/M and GMP-MLLAF9. (A) GFP⁺ cells in bone marrow of mice injected with floxed β -catenin (β cat^{loxP/loxP}) or β -catenin-deficient (β cat^{-/-}) KLS-HoxA9/M cells at 18 hours, 1 month, or 4 months after transplantation. HoxA9/M- β cat^{loxP/loxP} KLS cells were transduced with a retroviral vector encoding Cre recombinase to generate β cat^{-/-} KLS-HoxA9/M cells or an empty vector to generate control β cat^{loxP/loxP} KLS-HoxA9/M cells. After selection, 1×10^6 infected cells were transplanted into sublethally irradiated recipients ($n = 5$ recipients in

each group). Mice were sacrificed at the indicated time points to assess for GFP⁺ cells. (B) Survival curves of mice transplanted with β cat^{-/-} KLS-HoxA9/M cells, β cat^{loxP/loxP} KLS-HoxA9/M cells, and empty vector or Cre-infected WT KLS-HoxA9/M (KLS) cells were sorted from WT C57BL/6 mice and empty vector or Cre-infected WT KLS-HoxA9/M leukemia development; $n = 10$ mice for each group). (C) Survival curves of mice transplanted with β cat^{-/-} GMP-MLLAF9 cells and β cat^{loxP/loxP} GMP-MLLAF9 cells ($n = 10$ mice for each group). P was determined by using the log-rank test.

Given the activation pattern of β -catenin in GMP-MLLAF9-derived and KLS-HoxA9/M-derived leukemias and its minimal activation in GMP-HoxA9/M-derived cells, we hypothesized that the inability of HoxA9/M to transform GMP might be due to its inability to sufficiently activate β -catenin. To test this, we transduced GMP-HoxA9/M cells with a retrovirus encoding a constitutively active form of β -catenin (β cat*) (Fig. 2A). Transplantation of GMP-HoxA9/M cells transduced with β cat* produced AML similar to KLS-HoxA9/M cells (Fig. 2B). Expression of β cat* in GMP cells did not induce leukemia (Fig. 2B).

We next tested the effect of indomethacin (Indo), a reversible COX inhibitor that blocks activation of the Wnt pathway by suppressing β -catenin expression (23). Exposure of LIC-enriched c-Kit^{high} cells to indomethacin reduced β -catenin levels in vitro (Fig. 3A), and mice treated with Indo showed a dramatic reduction in the c-Kit^{high} population after 7 days of treatment (Fig. 3B). We performed a limiting dilution assay using flow-sorted GFP+ cells from bone marrow of control and Indo-treated mice, which demonstrated a 100-fold decrease in LIC after indo treatment (Fig. 3C). Furthermore, Indo treatment of a fully developed MLL-AF9 leukemia partially suppressed β -catenin (fig. S8) and reduced LSC frequency (Fig. 3D). Thus, indomethacin treatment can suppress the LIC compartment that is critical for the development of AML and also influences established LSC, suggesting that AML is dependent on β -catenin signaling. However, the therapeutic effect is greater in situations of minimal disease (Fig. 3C) versus more extensive disease (Fig. 3D).

Next, we examined the ability of HoxA9/M to induce leukemia from β -catenin-deficient KLS cells using bone marrow from β cat^{loxP/loxP} mice (25). We transduced β cat^{loxP/loxP} KLS cells with HoxA9/M, sorted single cells (KLS-HoxA9/M β cat^{loxP/loxP}), and subsequently transduced the cells with a retroviral vector encoding either Cre recombinase or an empty vector. Cells transduced with Cre recombinase (KLS-HoxA9/M β cat^{-/-}) were devoid of wild-type (WT) β -catenin (fig. S9). These cells were injected into recipient mice, and we monitored expansion of KLS-HoxA9/M β cat^{loxP/loxP}, KLS-HoxA9/M β cat^{-/-}, and control cells in bone marrow. KLS-HoxA9/M β cat^{loxP/loxP} cells expanded efficiently in mouse bone marrow (Fig. 4A) and ultimately produced leukemia. However, loss of β -catenin led to impaired expansion, and KLS-HoxA9/M β cat^{-/-} cells ultimately lost their ability to expand in bone marrow (Fig. 4A). β cat* rescued the proliferative defect of KLS-HoxA9/M β cat^{-/-} cells (fig. S9). Additionally, β cat^{WT} KLS-HoxA9/M cells, β cat^{WT} KLS-HoxA9/M cells expressing Cre recombinase, and KLS-HoxA9/M β cat^{loxP/loxP} cells induced AML in all recipients with similar latencies, whereas KLS-HoxA9/M β cat^{-/-} cells were dramatically impaired in their ability to initiate AML (Fig. 4B). Three mice injected with KLS-HoxA9/M β cat^{-/-} cells did ultimately develop AML; how-

ever, in the two cases that were captured before the mouse succumbed to the disease there was incomplete excision of β -catenin (fig. S9). Finally, a similar experiment demonstrated a dramatic reduction in the ability of MLL-AF9 to induce leukemia from GMP (Fig. 4C).

We have shown that β -catenin is required for Hox-mediated transformation of HSC and MLL-AF9-mediated transformation of committed progenitor cells. Our data also indicate that the lack of β -catenin activation in normal GMP limits the ability of Hox genes to efficiently transform committed progenitor cells. This is in contrast to MLL-AF9, which activates sufficient β -catenin to transform GMP. Overall, these findings support the concept that activation or maintenance of HSC-associated developmental pathways in downstream myeloid cells is an important component of AML development. Given that β -catenin activity is crucial for HSC survival during fetal development, but not for adult HSC survival/proliferation (9, 11, 12), targeting the β -catenin pathway may be an attractive therapeutic avenue in this disease.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5973/1650/DC1
Materials and Methods
Figs. S1 to S9
References

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β_2 -Adrenergic Receptor Redistribution in Heart Failure Changes cAMP Compartmentation

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The β_1 - and β_2 -adrenergic receptors (β ARs) on the surface of cardiomyocytes mediate distinct effects on cardiac function and the development of heart failure by regulating production of the second messenger cyclic adenosine monophosphate (cAMP). The spatial localization in cardiomyocytes of these β ARs, which are coupled to heterotrimeric guanine nucleotide-binding proteins (G proteins), and the functional implications of their localization have been unclear. We combined nanoscale live-cell scanning ion conductance and fluorescence resonance energy transfer microscopy techniques and found that, in cardiomyocytes from healthy adult rats and mice, spatially confined β_2 AR-induced cAMP signals are localized exclusively to the deep transverse tubules, whereas functional β_1 ARs are distributed across the entire cell surface. In cardiomyocytes derived from a rat model of chronic heart failure, β_2 ARs were redistributed from the transverse tubules to the cell crest, which led to diffuse receptor-mediated cAMP signaling. Thus, the redistribution of β_2 ARs in heart failure changes compartmentation of cAMP and might contribute to the failing myocardial phenotype.

The β_1 and β_2 adrenergic receptors (β ARs) are heterotrimeric guanine nucleotide-binding protein (G protein)-coupled re-

ceptors found on the surface of cardiac muscle cells (cardiomyocytes). They mediate cardiac responses to catecholamine hormones through

their coupling to G_s proteins and to the production of the common second messenger cyclic adenosine monophosphate (cAMP) (1). Selective stimulation of these two receptor subtypes leads to distinct physiological and pathophysiological responses. β_1 ARs but not β_2 ARs stimulate the cAMP-dependent protein kinase (PKA)-mediated phosphorylation of phospholamban and cardiac contractile proteins (2), induce hypertrophy upon moderate overexpression (3, 4), and promote cardiomyocyte apoptosis (5, 6). In ventricular cardiomyocytes isolated from transgenic mice expressing a fluorescence resonance energy transfer (FRET)-based cAMP sensor, we recently showed that, upon local receptor stimulation, β_1 AR-induced receptor-mediated cAMP signaling propagated throughout the entire cell, whereas β_2 AR-cAMP responses were locally confined by an unknown mechanism (7). The spatial localization of the receptors and the compartmentation of

their signaling is thought to play a critical role in cardiac physiology and the development of cardiac disease, such as heart failure (8–12). However, the precise distribution of the β_1 and β_2 ARs in cardiomyocytes with respect to the highly organized sarcomeric structure of these cells and its potential functional implications are still elusive. As for most G protein-coupled receptors, immunocytochemical or electron microscopy detection of native β ARs receptors with antibodies is limited by the low expression level of these proteins and by insufficient antibody specificity.

Here, we present an alternative approach to determine both position and function of endogenous β AR subtypes, by combining scanning ion conductance microscopy (SICM) with measurements of cAMP production by FRET after local receptor stimulation (13). SICM is a nonoptical method (14) in which a nanopipette is used as a scanning probe for non-contact visualization of the three-dimensional surface topography of living cells (15, 16). SICM is based on measuring the changes in ion flow through the pipette positioned at the cell surface and allows resolution of the structural features of cardiomyocytes, such as Z-grooves, cell crests located between them, and transverse (T)-tubules (Fig. 1), with a resolution equal to the pipette's inner diameter

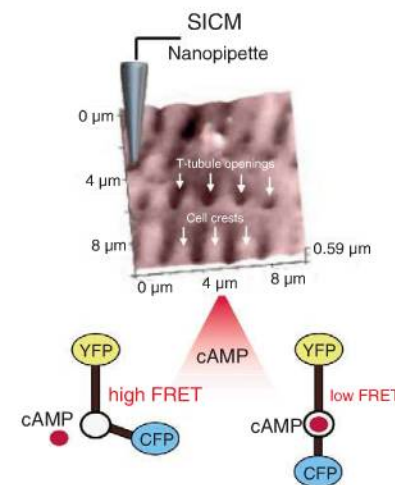
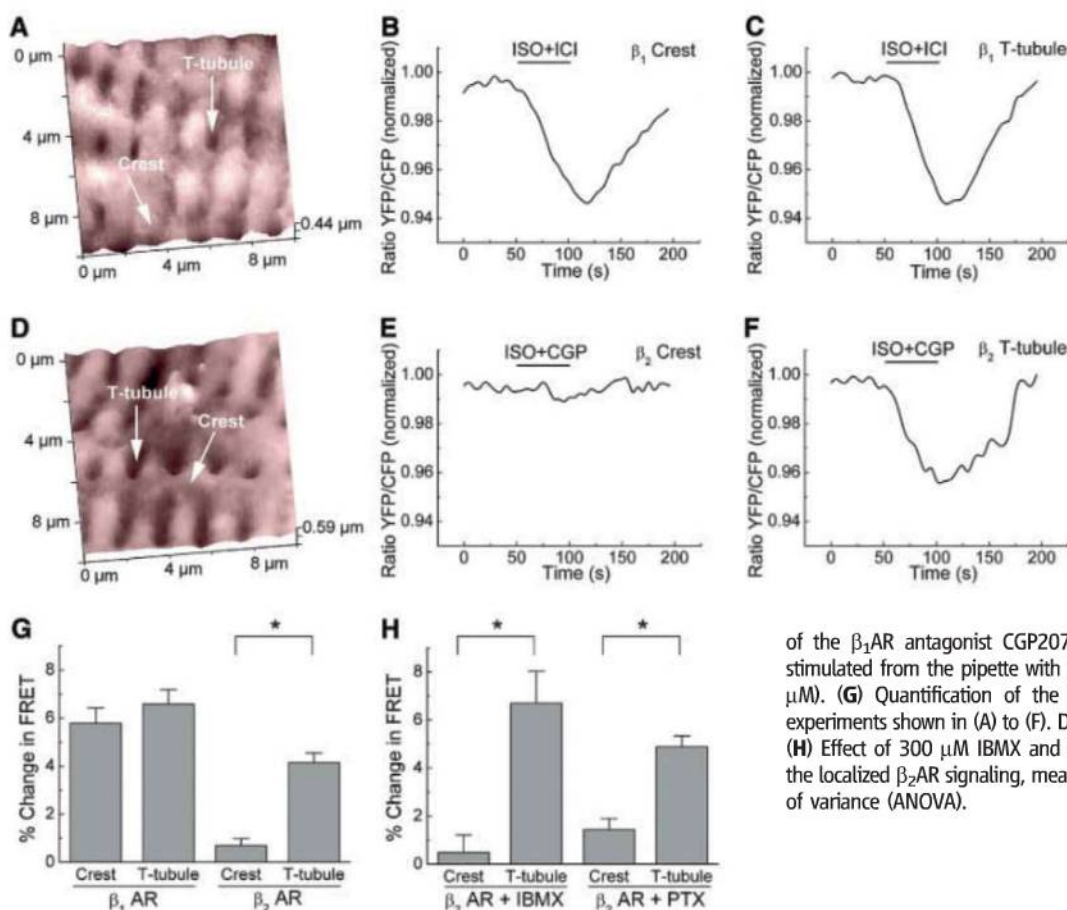


Fig. 1. Functional localization of β AR-induced cAMP signaling and the principle of the combined nanoscale SICM-FRET approach. Typical SICM image of a rat cardiomyocyte shows defined morphological structures that are used to position the nanopipette and to stimulate receptors in a highly localized fashion. Receptor activity is measured by monitoring the production of cAMP by Epac2-camps, a FRET-based cAMP sensor that changes its conformation and fluorescence properties upon activation—i.e., cAMP binding. YFP and CFP, yellow and cyan fluorescent proteins, respectively.



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(17), typically ~50 to 100 nm, as confirmed by electron microscopy. The SICM pattern of T-tubule distribution colocalized well with the T-tubular marker di-8-ANEPPS, a voltage-sensitive dye (fig. S1A).

We isolated ventricular cardiomyocytes from healthy adult rats, introduced into the cells an adenovirus vector encoding the cytosolic cAMP sensor Epac2-camps (18), and acquired an image of the cell surface topography by SICM. Next, we positioned the pipette onto various membrane regions of defined morphology and applied receptor ligands in a highly localized fashion. On selective local stimulation of β_1 and β_2 ARs, we measured cAMP synthesis and its accumulation in the cell cytosol by FRET microscopy (Fig. 1). Local stimulation was achieved by applying pressure to the pipette while constantly superfusing the cells with buffer solution (7). Pressure application resulted in the delivery of ~1.6 pL of the agonist solution onto the cell surface (fig. S1B) and did not cause any membrane deformations, as measured by SICM (fig. S1C). Local ligand application ensured activation of a small (≤ 500 nm) sarcolemmal area, as confirmed by labeling of the membrane with the fluorescent β AR ligand BODIPY-TMR-CGP12177 (fig. S1D). This area was in the range of the size of a T-tubule opening and well below the size of a sarcomere.

To study the localization of receptors and cAMP signals in cardiomyocytes isolated from healthy adult rat hearts, we locally stimulated β_1 and β_2 ARs either in the T-tubule or on the cell crest. Selective stimulation of β_1 ARs in both regions resulted in a robust decrease of FRET, reflecting the activation of cAMP synthesis by the receptors localized in different parts of the membrane (Fig. 2, A to C). In sharp contrast, β_2 -cAMP signals were observed only after local T-tubular stimulation, but not after stimulation at the cell crest (Fig. 2, D to F). We quantified changes in FRET (Fig. 2G) after local receptor stimulations and investigated whether the absence of the β_2 AR signal on the cell crest results from higher rates of local cAMP degradation by phosphodiesterases (PDEs) (8) or from coupling of the β_2 AR to G_i proteins (19, 20) in this particular compartment. Inhibition of PDEs with 3-isobutyl-1-methylxanthine (IBMX) led to slightly higher cAMP levels after stimulation in the T-tubules but did not produce any signals after stimulation in the cell crest. Likewise, G_i -protein inactivation with pertussis toxin (PTX) did not change the localized pattern of the β_2 AR signals (Fig. 2H). These data indicate that, in normal cardiomyocytes, β_2 ARs are exclusively localized to the T-tubules, whereas β_1 ARs are present in both the cell crests and the T-tubules. To exclude the possibility that the absence of the β_2 AR signal on the cell

crest was due to the lower expression level of the β_2 versus β_1 ARs in cardiomyocytes, we partially blocked β_1 ARs by applying 40 nM of the β_1 AR antagonist CGP20712A, so that the β_2 and β_1 stimuli were of equal strength (7). Even in this case, β_1 AR signals were detected equally well in the T-tubules and on the cell crest (fig. S2). We also tested the possibility that β_3 ARs might blunt cAMP production, as reported for neonatal cardiomyocytes (21). Addition of the selective β_3 AR antagonist SR59230A did not cause any change in FRET signals induced by local selective β_1 and β_2 AR stimulations (fig. S3).

To analyze individual effects of β_1 and β_2 ARs, we studied adult cardiomyocytes isolated from either β_2 or β_1 AR knockout (KO) mice transgenically expressing the FRET-based cAMP sensor. We first confirmed that cardiomyocytes from wild-type mice exhibited a pattern of β_1 and β_2 AR distribution similar to the pattern we noted for healthy rat cardiomyocytes (fig. S4, A to D). We then showed that cardiomyocytes from wild-type and KO mice had a similar surface morphology (fig. S4, B, F, and I). Consistent with the rat data, we found that β_1 AR-cAMP signals in the β_2 AR-KO myocytes were detectable on β_1 AR stimulation in both cell crests and T-tubular regions, whereas β_2 ARs in β_1 AR-KO cells showed locally confined cAMP signals in the T-tubules (fig. S4, G, K, and L).

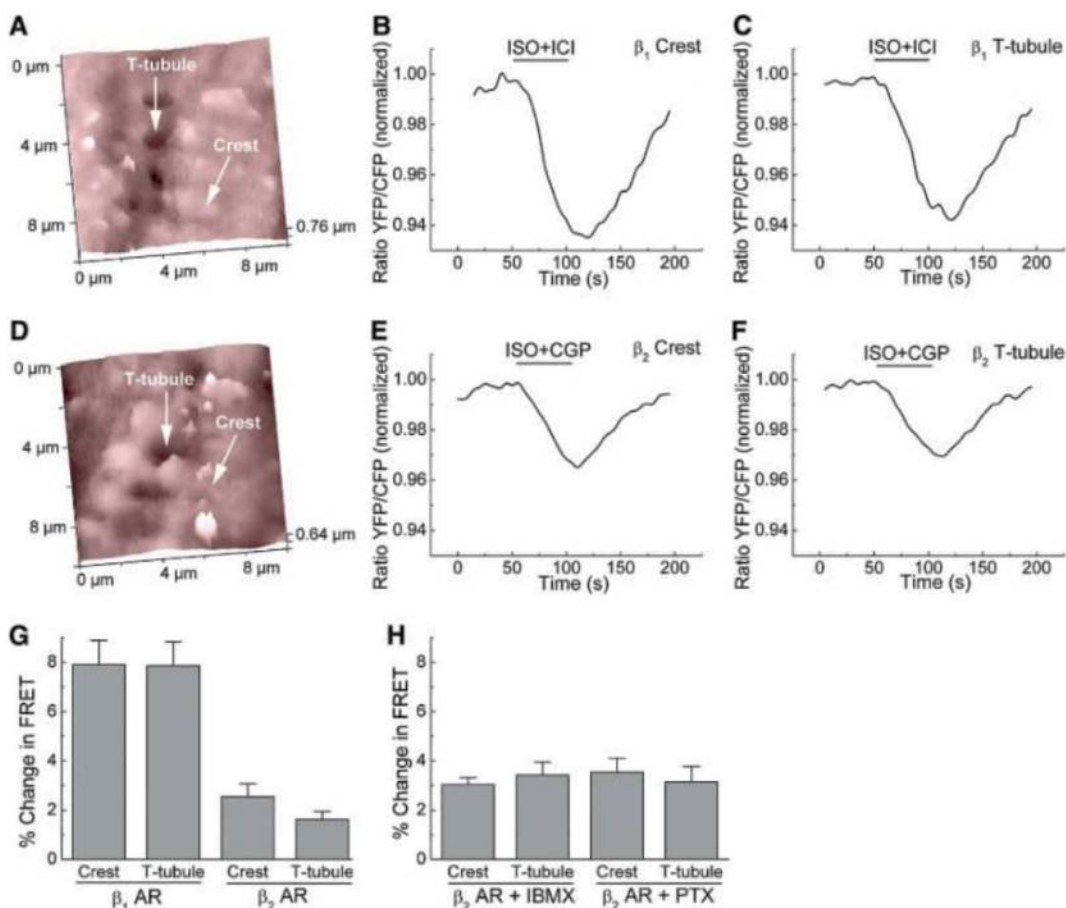


Fig. 3. β AR-induced cAMP signals in cardiomyocytes from rats with chronic heart failure. (A to C) SICM image and corresponding FRET ratio traces after local β_1 AR stimulation in the detubulated area (B) and in the T-tubule (C). The cells were superfused and stimulated from the nanopipette as described in Fig. 2. (D to F) SICM image and corresponding ratio traces after local β_2 AR stimulation in the detubulated area (E) and in the T-tubule (F). (G) Quantification of the changes in cAMP-FRET ratios from experiments shown in (A) to (F). Data are means $\pm$ SEM ($n \geq 8$). (H) Effect of 300 μ M IBMX and of pretreatment with 1.5 μ g/ml PTX on the localized β_2 AR signaling, means $\pm$ SEM ($n \geq 5$).

We next studied whether β AR localization was altered in a rat model of chronic heart failure induced by myocardial infarction. We have shown severe functional deficits in this model both at the whole-organ and cellular levels, with cardiomyocytes demonstrating marked structural abnormalities, including an extensive loss of the T-tubules (17) (Fig. 3, A and D), and β AR desensitization, a hallmark of cardiac failure (fig. S5). We analyzed the distribution of the β_1 AR- and β_2 AR-mediated cAMP signaling in experiments similar to those performed with the healthy cells (Fig. 2). Selective local stimulation of β_1 ARs led to cAMP signals equally detectable in T-tubules and in the nontubular regions (Fig. 3, A to C). Note that selective local β_2 AR-stimulation of failing cells revealed cAMP signals originating not only from the T-tubules, but also from the receptors locally activated in the nontubular areas (Fig. 3, D to F). The amplitudes of these β_2 AR-cAMP signals were smaller than the β_2 AR signals from the T-tubules of healthy cells [$P < 0.01$, by analysis of variance (ANOVA), compare Fig. 3G and Fig. 2G], but the cAMP production was clearly detectable after local stimulation of different sarcolemmal regions (Fig. 3, E to G), which

suggested that, in heart failure, β_2 ARs redistribute from the T-tubules to the cell crest. Once again, modulation of PDE activity and G_i coupling had only minor effects (Fig. 3H). We observed a similar redistribution of β_2 ARs in rat and in β_1 AR-KO mouse cardiomyocytes after artificial formamide-induced detubulation, which suggested that it is the loss of T-tubules in disease that drives the selective redistribution of the β_2 AR subtype (fig. S6).

We explored the spatial organization of cAMP signaling from differentially localized β ARs in normal and failing cardiomyocytes by analyzing the distribution of the FRET signals in different parts of the cell cytosol after local β_2 AR stimulation. To validate the measurements of cAMP diffusion using a soluble sensor protein, we confirmed that the diffusion of the sensor itself did not change upon receptor stimulation (fig. S7, A to C). β_2 AR-induced cAMP signals were highly locally confined when this receptor was activated in the T-tubules of healthy cells (Fig. 4, A and E). Note that stimulation of β_2 ARs in detubulated areas of failing cardiomyocytes produced diffuse cAMP signaling that propagated throughout the entire cytosol (Fig. 4, B and E), similar in behavior to the β_1 AR-generated signal (fig. S7D).

We hypothesized that cAMP buffering by the cAMP-dependent protein kinase (PKA) (22) might contribute to the localized β_2 AR-cAMP signaling. It was noteworthy that immunostaining of the kinase's RII subunit revealed a loss of the striated PKA localization pattern in cells from rats with heart failure (Fig. 4, A and B), although the pattern of potential PKA targeting structures, such as sarcoplasmic reticulum (SR), was not distorted (fig. S7F). Treatment of healthy cells with the Ht31 peptide, which blocks the interaction between RII subunits and A-kinase-anchoring proteins (10, 23), led to disruption of the T-tubular localization of the PKA and to propagation of β_2 AR-cAMP signals throughout larger parts of the cell (Fig. 4, C to E). This finding suggests that the cAMP produced on stimulation of β_2 ARs in the T-tubules may be locally confined by the interaction with localized PKA molecules enriched in this compartment. In addition, activation of PKA by cAMP may lead to a local increase in PDE4 activity, which provides a negative-feedback control of the cAMP levels (8). To test this possibility, we blocked PDE4 by rolipram and observed that β_2 AR-cAMP signals now propagated from locally activated T-tubules (fig. S7E). Thus, local buffering of cAMP by PKA and activation of PDE4 by this

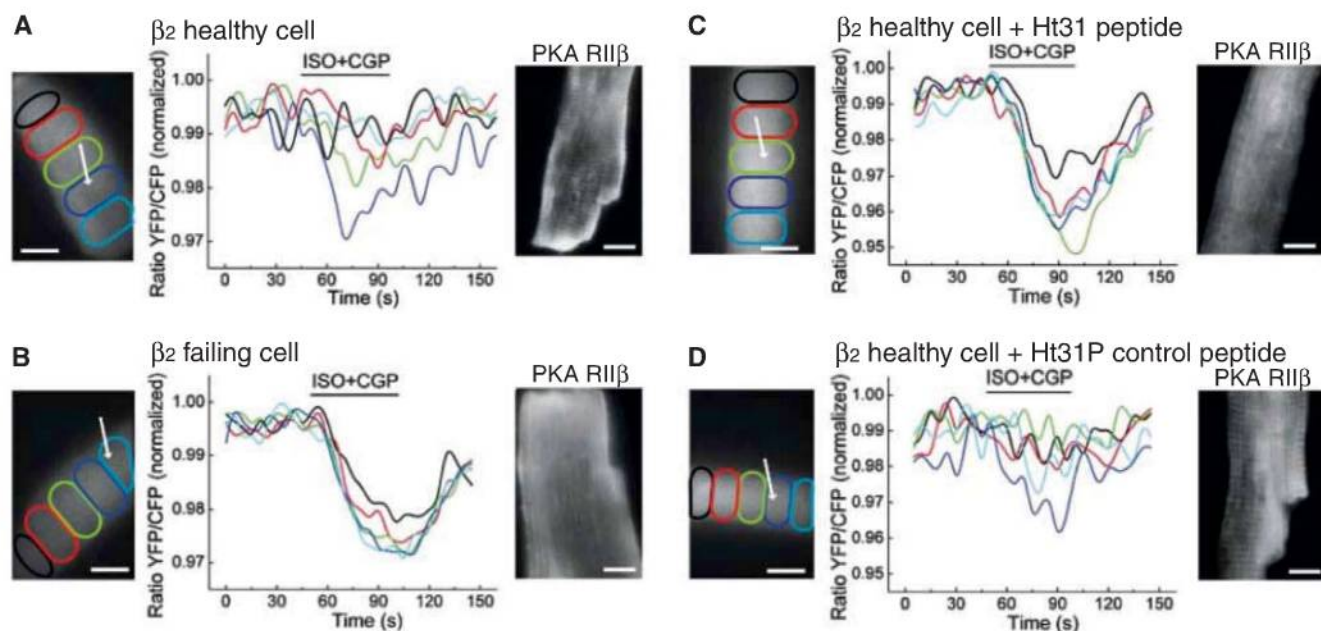
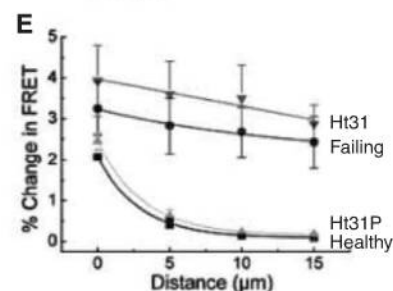


Fig. 4. Differences in spatial organization of cAMP signaling in cardiomyocyte from healthy rats and rats with heart failure. (A) Local stimulation of β_2 ARs in the T-tubule of a healthy cardiomyocyte, as described in Fig. 2F, demonstrates a locally confined cAMP response detectable only at the site of stimulation (arrow, $n \geq 7$). The graph shows FRET ratio changes measured in five different regions marked with colored ovals (left). (B) β_2 AR stimulation in a detubulated area of a failing cell induces a global cAMP signal propagating throughout the cytosol ($n \geq 7$). Note the loss of striated PKA localization pattern in failing cells (right). (C and D) Treatment of healthy cells with 50 μ M Ht31 (C) but not with control Ht31P peptide (D) for 40 to 60 min induces the disruption of the striated PKA localization (right) and propagating cAMP signals following local β_2 AR stimulation ($n = 5$). Scale bars in (A) to (D), 20 μ M. (E) Quantification of cAMP signal propagation for experiments presented in (A) to (D). Data are means $\pm$ SEM.



kinase contribute to the confinement of β_2 AR-cAMP signaling.

One possible mechanism that might be responsible for T-tubule-selective β_2 AR localization is the interaction of this receptor with lipid rafts (11). To investigate the role of these structures in the β_2 AR localization and signaling, we performed SICM-FRET experiments in rat cardiomyocytes after membrane cholesterol depletion by methyl- β -cyclodextrin (M β CD). M β CD treatment did not cause any loss of T-tubules but induced β_2 AR redistribution and propagation of β_2 AR-cAMP signals from the crest of the cell (fig. S8), which suggested that the interaction of β_2 ARs with cholesterol-rich membrane domains is important for normal β_2 AR localization and signal compartmentation.

On the basis of the observed distribution of β_1 and β_2 AR signals in failing versus healthy cardiomyocytes, we propose a model in which the compartmentation of the β_2 AR-cAMP signaling changes in heart failure (fig. S9). Redistribution of the β_2 AR from the T-tubules to the cell crest in failing cardiomyocytes and the loss of proper PKA localization, observed also in human heart failure (24), results in uncoupling of the β_2 ARs from the localized pools of PKA that are responsible for the compartmentation of the β_2 AR-cAMP signaling. Thus, in failing cells, activation of β_2 ARs leads to cell-wide cAMP signal propagation patterns, similar to the patterns observed for β_1 ARs (compare Fig. 4B and fig. S7D). Upon redistribution of the receptor, β_2 AR signaling may lose its normally cardioprotective properties and may acquire the characteristics of the β_1 AR response, thus contributing to the heart failure phenotype. It has been previously noted that β_2 AR signals in ventricular myocytes from failing human hearts and animal heart failure models had functional effects more characteristic of the β_1 AR (25, 26). The propagating β_2 AR-cAMP gradients that we observed in failing cardiomyocytes and in normal cardiomyocytes treated with rolipram or M β CD are compatible with the ability of β_2 ARs to induce phospholamban and troponin I phosphorylation that was reported in these experimental settings and that is associated with arrhythmogenic effects of the β_2 AR in heart failure (27–30).

In summary, using the SICM-FRET technique, we were able to functionally localize β_1 ARs and β_2 ARs to the surface structures of adult ventricular cardiomyocytes and to uncover the mechanisms leading to the abnormal cAMP compartmentation in heart failure. These findings should provide a deeper understanding of this cardiac disease and facilitate the development of new therapeutic strategies.

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Loss of Rap1 Induces Telomere Recombination in the Absence of NHEJ or a DNA Damage Signal

Agnel Sfeir,* Shaheen Kabir,* Megan van Overbeek,† Giulia B. Celli, Titia de Lange‡

Shelterin is an essential telomeric protein complex that prevents DNA damage signaling and DNA repair at mammalian chromosome ends. Here we report on the role of the TRF2-interacting factor Rap1, a conserved shelterin subunit of unknown function. We removed Rap1 from mouse telomeres either through gene deletion or by replacing TRF2 with a mutant that does not bind Rap1. Rap1 was dispensable for the essential functions of TRF2—repression of ATM kinase signaling and nonhomologous end joining (NHEJ)—and mice lacking telomeric Rap1 were viable and fertile. However, Rap1 was critical for the repression of homology-directed repair (HDR), which can alter telomere length. The data reveal that HDR at telomeres can take place in the absence of DNA damage foci and underscore the functional compartmentalization within shelterin.

The shelterin subunit TRF2 is involved in the repression of the telomeric DNA damage response (1). Deletion of TRF2 results in activation of the ataxia telangiectasia mutated (ATM) kinase and telomere fusions mediated by nonhomologous end joining (NHEJ). TRF2 also contributes to the repression of homology-directed repair (HDR), which can create undesirable telomeric sister chromatid exchanges (T-SCEs). HDR at telomeres occurs in Ku70/80-deficient cells upon deletion of either TRF2 or the two POT1 proteins (2, 3).

The repression of ATM signaling, NHEJ, and HDR by TRF2 could potentially involve Rap1, which depends on TRF2 for its stable expression and recruitment to telomeres (4, 5). Telo-

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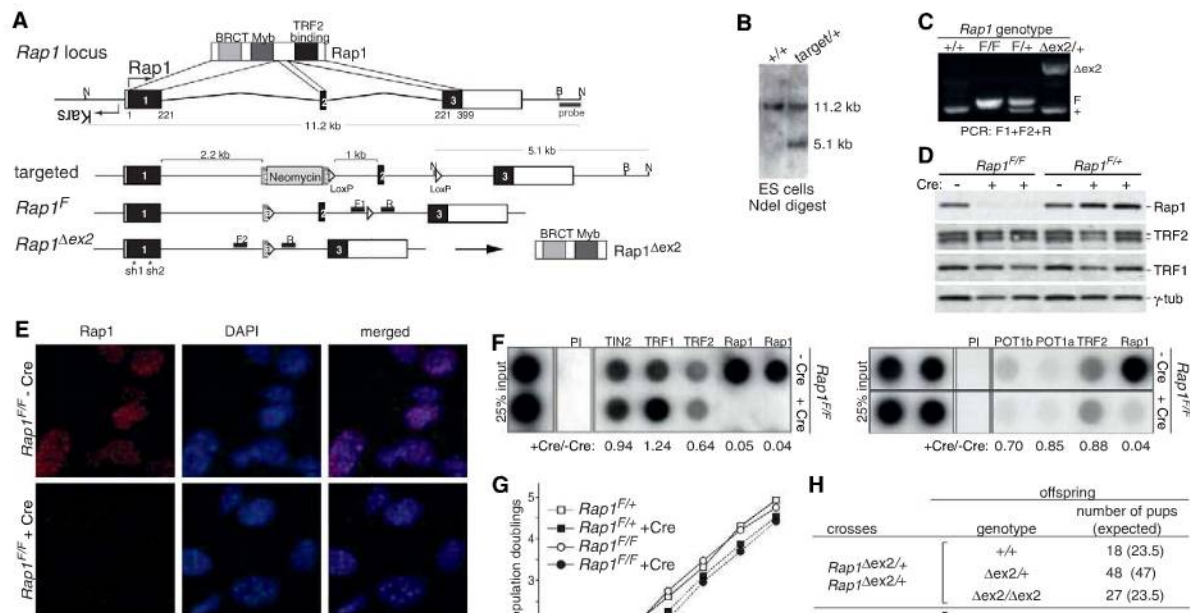


Fig. 1. Deletion of *Rap1* does not affect cell and organismal viability. (A) Schematic of *Rap1*, the mouse *Rap1* (*Terf2ip*) locus, the targeting construct, the floxed allele, and the $\Delta ex2$ allele. N, Nde I; B, Bam HI; F1, F2, and R, polymerase chain reaction primers. *Rap1* shRNAs are shown at the bottom. At right, *Rap1* ^{$\Delta ex2$} -encoded protein. (B) Genomic blot of Nde I-digested DNA from embryonic stem (ES) cells. Probe is in (A). (C) Genotyping of tail DNAs. Primers are in (A). (D) Immunoblots for *Rap1* (Ab1252), TRF2 (Ab1254), and TRF1 (Ab1449) from *Rap1*^{F/F} and *Rap1*^{F/+} MEFs 5 days after Hit-and-run (H&R)-Cre (first lane) or pWZL-Cre (second lane). (E) Loss of *Rap1* IF signal from Cre-treated (day 5) *Rap1*^{F/F} MEFs. Red, *Rap1*;

green, telomeric fluorescence in situ hybridization (FISH); blue, DNA (DAPI, 4',6'-diamidino-2-phenylindole). (F) Telomeric ChIPs on Cre-treated (day 5) *Rap1*^{F/F} MEFs. Numbers represent ratios of percent telomeric DNA in the ChIPs [preimmune (PI) signal subtracted] on cells with (+) and without (-) Cre. (G) Proliferation of SV40LT-immortalized *Rap1*^{F/F} and *Rap1*^{F/+} MEFs infected as indicated. (H) Offspring from *Rap1*^{Δex2/+} and *Rap1*^{Δex2/Δex2} intercrosses.

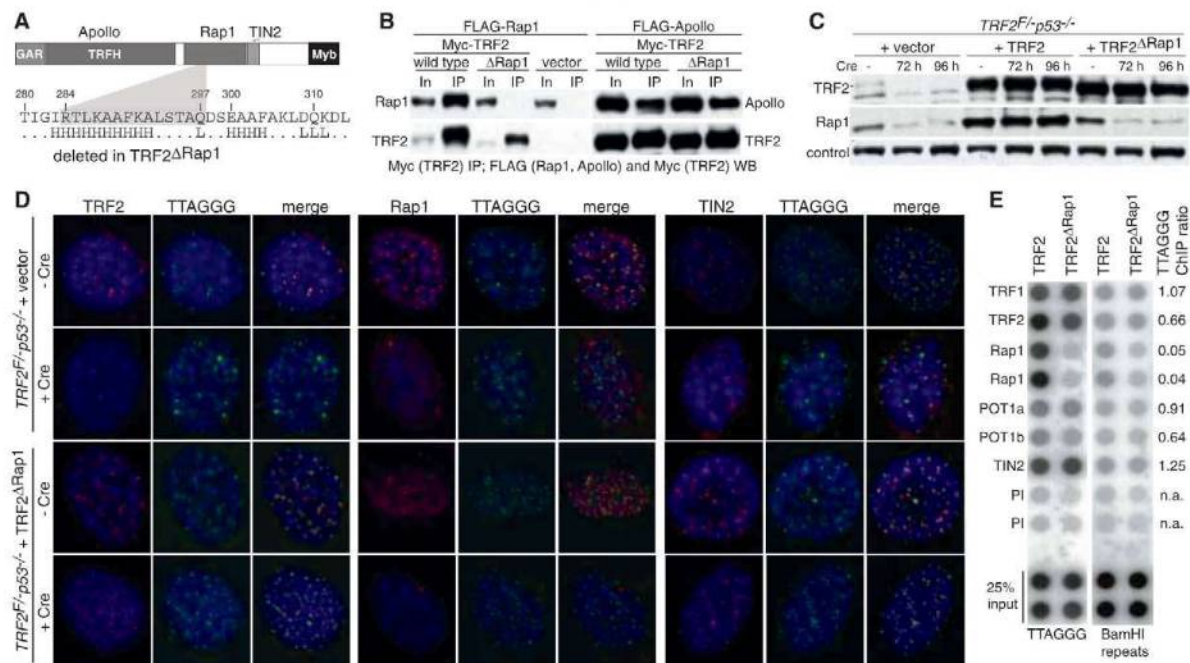
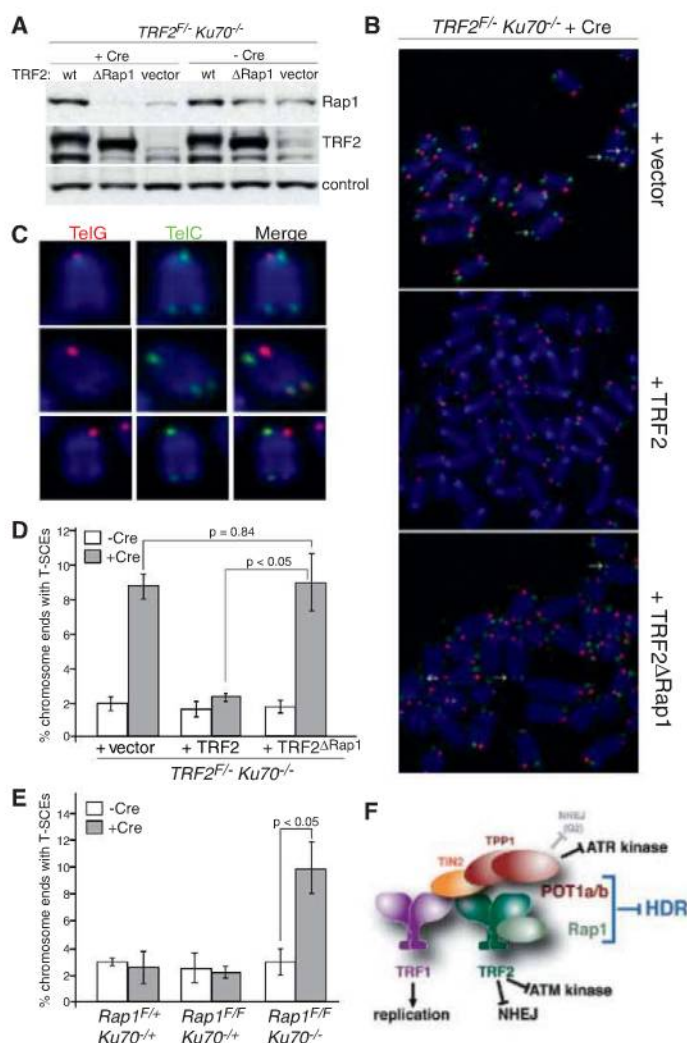


Fig. 2. A TRF2 mutant deficient for *Rap1* binding. (A) The *TRF2*^{ΔRap1} mutant. H, predicted helix. Amino acid residues: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; Q, Gln; R, Arg; S, Ser; and T, Thr. (B) Coimmunoprecipitation of Myc-TRF2 or Myc-TRF2^{ΔRap1} with FLAG-Rap1 or FLAG-Apollo from cotransfected 293T cells. In, 2.5% of input. (C) Immunoblots for TRF2 and Rap1 from *TRF2*^{F/p53-/-} MEFs expressing the indicated alleles at 72 and 96 hours after H&R-Cre. (D) IF-FISH to monitor

TRF2, *Rap1*, and *TIN2* at telomeres in *TRF2*^{F/p53-/-} MEFs expressing *TRF2*^{ΔRap1} or vector control at day 4 after Cre treatment. (E) Telomeric ChIP of *TRF2*^{F/p53-/-} MEFs expressing *TRF2* or *TRF2*^{ΔRap1} at day 7 after Cre treatment. Duplicate dot blots were probed for telomeric DNA or the dispersed Bam HI repeats. ChIP ratios represent the percentage of telomeric DNA recovered in *TRF2*^{ΔRap1}- versus *TRF2*-expressing cells calculated as in Fig. 1.

Fig. 4. Rap1 is a repressor of telomere recombination. (A) Rap1 and TRF2 from *TRF2^{F/F}-Ku70^{-/-}* MEFs expressing TRF2, TRF2^{ΔRap1}, or vector control analyzed 4 days after Cre treatment. (B) Chromosome orientation (CO) FISH analysis on cells as in (A). Arrows: T-SCEs. (C) Enlarged T-SCE events in Cre-treated *TRF2^{F/F}-Ku70^{-/-}* MEFs expressing TRF2^{ΔRap1}. (D) Quantification of T-SCEs as assessed in (B). Bars represent the mean $\pm$ SD from three independent experiments ($n > 1100$ chromosome ends each). *P* values are based on Student's two-tailed *t* test. (E) Quantification of T-SCEs as assessed in (B) in cells of the indicated Rap1 and Ku70 status. Method as in (D). Error bars: SEM (*Rap1^{F/F}-Ku70^{-/-}* and *Rap1^{F/F}-Ku70^{-/-}*) or SD (*Rap1^{F/F}-Ku70^{-/-}*). (F) The functions of shelterin components. See text for details.



region [amino acids 260 to 360 (5)] was conserved in TRF2 orthologs but not in TRF1 (fig. S2, A and B). Two mutations in this region (A289S and F290S) reduced the interaction between Rap1 and TRF2 in coimmunoprecipitation experiments (fig. S2C). To generate TRF2^{ΔRap1}, we deleted amino acids 284 to 297 (Fig. 2A). TRF2^{ΔRap1} failed to bind to Rap1 in coimmunoprecipitation experiments, whereas it retained its interaction with Apollo (Fig. 2B). Wild-type TRF2 and TRF2^{ΔRap1} were expressed in *TRF2^{F/F}-p53^{-/-}* MEFs, and the endogenous TRF2 was removed with Cre (Fig. 2C). Although TRF2^{ΔRap1} localized to telomeres efficiently, IF and ChIP indicated that the telomeres lacked Rap1, and the overall abundance of Rap1 in the cells was reduced (Fig. 2, C to E, and fig. S3A). Other shelterin components were affected to an extent (less than twofold; Fig. 2, D and E) that is not expected to be functionally important because heterozygous MEFs and mice lacking one copy of *TRF1*, *TPP1*, *TRF2*, or *POT1a/b* display no telomere defect. Consistent with the viability of *Rap1^{Δex2/Δex2}* cells, cells expressing TRF2^{ΔRap1} proliferated at the same rate as cells expressing wild-type TRF2 (fig. S3B).

Rap1^{Δex2/Δex2} cells did not show telomere dysfunction-induced foci [TIFs (13)], which are telomeric DNA damage foci that report on ATM and/or ATR (ataxia telangiectasia and Rad3-related) signaling at chromosome ends, and phosphorylation of Chk1 and Chk2 was not evident (Fig. 3, A to C). Further depletion of *Rap1* mRNA with an shRNA also failed to elicit a DNA damage signal in *Rap1^{Δex2/Δex2}* cells (Fig. 3B). Consistent with these results, TRF2^{ΔRap1} was equivalent to wild-type TRF2 in its ability to repress TIFs in cells lacking the endogenous TRF2 (Fig. 3D). The mutant form of TRF2 also repressed the induction of Chk2 phosphorylation to the same extent as wild-type TRF2 (Fig. 3E). The low level of Chk2-P observed in Cre-treated TRF2- and TRF2^{ΔRap1}-expressing cells is likely due to Cre-induced DNA damage, because the phosphorylation of Chk2 was diminished when using a version of Cre that eventually disappears from the cells due to self-deletion (fig. S4). Furthermore, telomere fusions were not induced by deletion of *Rap1*, and TRF2^{ΔRap1} had the same ability as wild-type TRF2 to repress NHEJ at telomeres (Fig. 3, F to H). However, because NHEJ of telomeres

lacking TRF2 requires active DNA damage signaling (14), the lack of telomere fusions could be due to the lack of ATM or ATR activation. We therefore used a TPP1 shRNA to activate ATR kinase signaling at telomeres. This approach previously resulted in the reactivation of NHEJ at telomeres of cells lacking both TRF2 and ATM (14). Despite the telomeric ATR kinase signal elicited by the TPP1 shRNA (Fig. 3, B and D), Rap1 removal from telomeres did not induce their fusion (Fig. 3, G and H).

Thus, Rap1 does not appear to be required in the repression of either NHEJ or ATM kinase signaling, explaining why the deletion of Rap1 does not curb cellular or organismal viability. In addition, Rap1 was not required for the maintenance of several other features of mouse telomeres, including the maintenance of telomere length over three generations of mouse breeding and in cultured cells, the amount of single-stranded telomeric DNA, the telomeric nucleosomal organization, the methylation of telomeric H3K9, and the abundance of telomeric transcripts [TERRA (15)] (fig. S5).

HDR threatens telomere integrity because unequal T-SCEs can change telomere lengths. T-SCEs are most frequent when either *TRF2* or *POT1a/b* are deleted from Ku-deficient cells (2, 3), although low frequency of T-SCEs have been reported for *POT1a* deficiency alone (16). To determine whether Rap1 was required for TRF2-mediated repression of T-SCEs, we introduced TRF2^{ΔRap1} into SV40LT-immortalized *TRF2^{F/F}-Ku70^{-/-}* MEFs, which display frequent T-SCEs upon deletion of *TRF2* with Cre (2) (Fig. 4). Whereas the telomeric exchanges were repressed by wild-type TRF2, TRF2^{ΔRap1} failed to block the telomeric HDR (Fig. 4, A to D). The frequency of T-SCEs was the same whether the cells expressed TRF2^{ΔRap1} or no TRF2. Furthermore, T-SCEs were induced by Cre-mediated deletion of Rap1 from *Rap1^{F/F}-Ku70^{-/-}* cells (Fig. 4E). The T-SCEs occurred despite the absence of TIFs in cells lacking both Ku70 and telomeric Rap1 (fig. S6).

These data indicate that Rap1 functions at mouse telomeres to repress HDR, which has the potential for generating shortened telomeres and can promote telomerase-independent telomere maintenance. Rap1 appears to be an adaptor protein: Its C terminus serves to anchor the protein in shelterin; its BRCT domain, when dimerized in the shelterin complex, could bind a phosphorylated target protein; and the surface charge of its Myb-type motif makes it a third potential protein-interaction domain (17). As adaptors, the Rap1 orthologs could fulfill diverse functions in different organisms, because alterations in one of the protein-interaction domains could endow Rap1 with a new binding partner and thus instigate a new function.

These results underscore the functional compartmentalization within shelterin (Fig. 4F), which contains at least four subunits dedicated to distinct functions. The replication of telomeric

DNA is facilitated by TRF1 (18), and TPP1/POT1 are required for the repression of ATR signaling (1, 19). TRF2 is the predominant repressor of both ATM signaling and NHEJ, and the current data show that these functions of TRF2 do not require Rap1. Finally, our results identify a fifth component of shelterin, Rap1, as an important repressor of HDR. Repression of HDR also requires TPP1/POT1 because removal of either Rap1 or POT1a/b result in telomere recombination. In a parallel pathway, Ku70/80 inhibits HDR, but it has not been established whether this function is telomere specific (2). This separation of function revealed that telomeres can undergo HDR without being detected by the ATM and ATR kinase pathways. When HDR takes place at telomeres lacking TRF2 or POT1a/b, DNA damage signaling results in the formation of TIFs. In the case of Rap1 removal, however, the telomeres lack detectable TIFs, yet are susceptible to HDR. Thus, consistent with the telomere recombination events in yeast lacking both Mec1 and Tel1 (20), the

formation of DNA damage foci at telomeres is not a prerequisite for HDR.

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Supporting Online Material

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The corrected Libra 200 transmission electron microscopes (TEM) come in two configurations: the Libra 200 CESTEM is based on the energy-filter version of the 200 kV Libra TEM with a corrector for spherical aberrations of the objective lens. Through the use of this corrector, image resolution below 0.7 Å can be achieved. Applications that can benefit from this development include imaging of interfaces in semiconductors or solar cells, grain boundaries in steel alloys, and damage induced by nuclear radiation in shielding materials. In these applications, the control of the material at the atomic scale is necessary for in-depth understanding of the underlying physical or chemical processes and to guarantee the functionality of these devices. Another advantage of the corrector is the ability to reduce the acceleration voltage down to 80 kV and still achieve resolutions below 1 Å, which reduces beam damage for analysis of sensitive materials like carbon nanotubes.

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DRUG DISCOVERY DATABASE

The Collaborative Drug Discovery (CDD) database is a novel web-based platform to archive and mine a broad range of objects. The database allows a broad array of data types to be archived and kept private and secure. The data can be shared selectively among colleagues or openly on the Internet in standardized formats. The CDD platform incorporates physical chemical calculations, similarity and substructure searching, and Boolean search and save capabilities. It can handle diverse data files from instruments and individual experiments as well as CSV and SDK file-convertible formats.

Collaborative Drug Discovery

For info: 650-219-4153 | www.collaborativedrug.com

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Science Careers

From the journal *Science*

POSITIONS OPEN



INNATE IMMUNITY TENURE-TRACK FACULTY POSITION

Department of Microbiology and Immunology
The University of Texas Medical Branch

The Department of Microbiology and Immunology at the University of Texas Medical Branch invites applications for a tenure-track position at the **ASSISTANT/ASSOCIATE PROFESSOR** level who will work in collaboration with Sandia National Laboratories to develop a strong, basic science research program that extends our efforts in biomarker discovery, diagnostics, therapeutics, and vaccine development for biodefense and emerging infectious diseases. This is a remarkable opportunity to unite the complementary infectious disease expertise of UTMB and the Galveston National Laboratory with the foundational science and technology expertise of Sandia. Candidates with interests in research on virus-induced signaling and innate immune mechanisms that can be applied to BSL-3/4 agents are encouraged to apply. We are seeking a scientist that has, or can develop success in competing for, research funding and who has a strong publication record. UTMB, as home to the National Institute of Allergy and Infectious Diseases-supported Western Regional Center of Excellence in Biodefense and Emerging Infectious Diseases and the Galveston National Laboratory, offers the successful candidate outstanding opportunities for collaborations with more than 150 scientists focusing on infectious disease/immunology research, unique BSL-3 and BSL-4 containment laboratories, and a broad array of state-of-the-art research core facilities. We offer an attractive startup package, modern laboratory space, and a highly interactive environment. For further information about the Department visit **website: <http://microbiology.utmb.edu/>**. Interested candidates should send curriculum vitae, a summary of past accomplishments, future research plans, and the names and contact information of four references electronically to **e-mail: nherzog@utmb.edu** by May 31, 2010.

UTMB hires only persons authorized to work in the United States. UTMB is an Affirmative Action/Equal Opportunity Employer.



The Whitaker Cardiovascular Institute of Boston University Medical School, **website: <http://www.bumc.bu.edu/busm-wci>**, is seeking applicants for its NIH-funded Multidisciplinary Training in Cardiovascular Research Program. Applicants should have an M.D. or Ph.D. and should be seeking a training position with a cardiovascular interest for at least two years. Contact **Nancy Clinton, e-mail: ncClinton@bu.edu** for an application. Due to federal regulations, *all applicants must have at least green card status at time of application. Boston University is an Equal Opportunity Affirmative Action Employer.*

The Center for Basic and Translational Obesity Research at Children's Hospital Boston is recruiting a translational physician-scientist at the **ASSISTANT PROFESSOR** level. Ideal candidate is Board-certified in a field related to pediatric obesity, with success in obtaining funding, an excellent publication record, and a translational research program aiming to inform or accelerate the development or improvement of interventions/therapies. Electronically send curriculum vitae and three references to **e-mail: joel.hirschhorn@childrens.harvard.edu**. Children's Hospital Boston is an Equal Opportunity Employer and encourages women and minorities to apply.

POSITIONS OPEN

CHAIR

Biomedical Engineering at the
University of Florida

Applications and nominations are invited for the position of Chair of the J. Crayton Pruitt Family Department of Biomedical Engineering at the University of Florida. In addition to overseeing the operational management of the Department, responsibilities of the Chair include: (1) creating a compelling vision for the advancement of the Department; (2) facilitating both the professional and scholarly growth of the faculty, particularly the junior faculty; (3) ensuring cutting-edge education is provided to all trainees (undergraduate, graduate, postdoctoral, and researchers); (4) enhancing the working partnership with the leaders of UF colleges and departments, the UF and Shands Health Sciences Center, and UF centers and institutes; (5) recruiting a diverse faculty and student body; and (6) increasing private and external funding opportunities for the Department. A Ph.D. degree and research and teaching experience in biomedical engineering are required. Management experience is preferred. Successful candidates should demonstrate academic credentials sufficient for appointment at the **FULL PROFESSOR** level.

The BME Department is endowed with \$20 million from the J. Crayton Pruitt Family and the state of Florida. It currently has 12 active tenure-track faculty members and more than 30 joint/affiliated faculty members and offers M.S. and Ph.D. degrees. The new undergraduate program will begin enrolling its first class of freshmen in fall 2010. The Department has recently completed its move into the new and state-of-the-art Biomedical Sciences Building, which is part of the UF and Shands Health Sciences Center complex and is adjacent to the College of Engineering. It is in the process of adding seven primary faculty lines in the next three to four years. Concurrent with these developments, the College of Engineering at the University of Florida has launched a new initiative to hire 20 new faculty in critical areas, including computation, sustainable infrastructure, information technology, nano/micro-technology, energy, and health care/biotechnology.

The University of Florida is the flagship campus of the state university system and offers excellence in research opportunities and facilities. The BME Department has a mandate to be the research and educational interface between the College of Engineering and the large and vibrant biomedical research community at and around UF, including the UF and Shands Health Sciences Center, the Evelyn F. and William L. McKnight Brain Institute, the NSF National High Magnetic Field Laboratory, the UF Shands Cancer Center, the new Nanoscience Institute for Medical and Engineering Technology, the Malcolm Randall Veterans Affairs Medical Center, a recently created institutional collaboration with the H. Lee Moffitt Cancer Center and Research Institute in Tampa, and the growing biotech industry in Gainesville and the state of Florida. With the awarding of an NIH Clinical and Translational Science Award (CTSA), there is enhanced opportunity for translational biomedical engineering research.

Curriculum vitae and letters of intent to apply should be electronically sent to **e-mail: chairsearch@bme.ufl.edu**. The Search Committee will begin reviewing applications starting April 15, 2010, and continue receiving applications until the position is filled. *The University of Florida is an Affirmative Action/Equal Opportunity Employer and women and minorities are strongly encouraged to apply.*

TENURE-TRACK ALZHEIMER'S POSITION

Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh, seeks an established, full-time faculty member to conduct laboratory research on Alzheimer's and related disorders. Applicants should have an M.D. or Ph.D. degree, a track record of extramural funding, and must be eligible to work in the U.S.A. Generous startup package is available and there is a possibility of an endowed chair. Applicants should electronically submit curriculum vitae and a brief statement of research interest to **J. Timothy Greenamyre, Chair, Search Committee, Department of Neurology, e-mail: neurologyinfo@upmc.edu**.

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NATIONAL HIGH MAGNETIC FIELD LABORATORY

Operated by Florida State University, University of Florida, Los Alamos National Laboratory
Florida State University, 1800 East Paul Dirac Drive, Tallahassee, Florida 32310

The National High Magnetic Field Laboratory (NHMFL) seeks a visionary leader as the Chief Scientist in Chemistry and Biology. The successful candidate will have a broad understanding of the most important problems in chemistry, biology, biomedicine, and biomedical engineering.

The NHMFL is the world's leading magnet laboratory, providing unique high-magnetic-field user facilities and hosting the research of approximately one thousand scientists annually. User research at the NHMFL spans condensed matter physics, materials research, chemistry, biology, biomedicine and biomedical engineering. The NHMFL consists of three campuses: Florida State University, the University of Florida and Los Alamos National Laboratory. It operates seven vibrant user programs: DC Magnetic Fields, Pulsed Magnetic Fields, High B/T (ultralow temperatures), Ion Cyclotron Resonance (ICR), Electron Magnetic Resonance (EMR), Nuclear Magnetic Resonance, and the Advanced Magnetic Resonance Imaging and Spectroscopy Facility (AMRIS). Each of these seven user programs is well established with strong leadership and staff. Major existing instrumentation of particular interest to chemistry and biology includes (1) a 21.1 T (900 MHz) ultra wide bore (105mm) NMR/MRI instrument, (2) an 11 T/40 cm MRI instrument, (3) a 36 T hybrid magnet designed for 1.5 GHz NMR and 1 THz EMR (to be completed in 2012), and (4) a recently funded 21 T / 105 mm horizontal bore magnet for ICR applications.

The NHMFL is presently leading a world-wide advance in superconducting magnet technology. The NHMFL has exploited major developments in high temperature superconducting (HTS) materials over the last two years to demonstrate the feasibility of superconducting magnets that will exceed 30 T. The NHMFL is making additional investments to realize high-homogeneity capabilities for HTS superconducting magnets. This represents a real revolution in magnet technology with the potential to transform Chem/Bio applications in ICR, EMR, NMR, and MRI utilizing high magnetic fields.

Another major initiative under development at the NHMFL is the proposed construction of "Big Light", a high-intensity light source that will provide bright, picosecond pulses of light that are tunable over the entire terahertz-to-infrared (THIR) frequency regime. This light source will be unique in the world and will be well matched to the energy and time scales relevant to much of the research conducted at the NHMFL. Consequently, high-field spectroscopy in the THIR regime represents another area with tremendous new opportunities for Chem/Bio research in the NHMFL user programs.

The NHMFL is looking for leadership to help broaden the operations and infrastructure funding base for the Chem/Bio user programs. The Chief Scientist in Chem/Bio will have a strong understanding of MRI, NMR, EMR, and ICR and will be an internationally recognized expert in at least one of these areas of technology. He/she will be expected to provide an expanded vision for the integrated use of existing and planned technologies to make a major impact in solving critical problems in chemistry & biology.

The successful candidate will have faculty appointments at both the University of Florida in Gainesville and Florida State University in Tallahassee. The primary institution and department are negotiable and will depend on the successful candidate's particular area of major research emphasis.

Interested candidates should send a curriculum vitae, cover letter describing interests and experience, and names and contact information of three references to Prof. Arthur S. Edison (aedison@ufl.edu), Chair, Chief Scientist in Chemistry and Biology Search Committee, National High Magnetic Field Laboratory. University of Florida and Florida State University are Equal Opportunity/Access/Affirmative Action Employers.

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Regional Centre for Biotechnology

an institution of education, training & research

(Established by the Dept. of Biotechnology, Govt. of India, under the auspices of UNESCO)

Career Opportunities for Chemists and Chemical Engineers

The Regional Centre for Biotechnology (RCB) invites scientists with innovative potential for research and teaching programmes interfacing biotech and chemical sciences as manifested in the fields of drug discovery, tissue engineering and synthetic biology. RCB, an international nodal point with local and global partnerships synergizing with UNESCO, will expand the opportunities to create world-class research and education. A description of the RCB and its mandates and projections can be found on the website <http://rcb.ac.in>.

Faculty appointments will be offered from entry to program coordinator levels depending on the quantum of experience and the quality of scientific productivity. Interested scientists are invited to send their curriculum vitae containing relevant details, list of publications and the names of three potential referees along with their electronic mail addresses to the **Executive Director, Regional Centre for Biotechnology, c/o National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi -110067, India**. An advance electronic copy of the bio-data may be sent to the e-mail address: rcb@nii.ac.in. Only short-listed candidates will be contacted for further discussions.



POSTDOCTORAL FELLOWSHIPS IN GENOMIC BIOLOGY

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

The Institute for Genomic Biology at the University of Illinois at Urbana-Champaign offers a number of postdoctoral fellowships for talented young scholars. IGB Fellows spend two to three years doing collaborative and independent research in one of several research themes at the Institute. Visit www.igb.illinois.edu/fellows for more information about the Institute, the research themes, and application procedures. The closing date for all positions is May 15, 2010. Fellows will be announced on or before July 1, 2010.

MOLECULAR BIOENGINEERING OF BIOMASS CONVERSION

We seek an individual with experience in metabolic engineering of industrially significant microbes (e.g. yeast, fiber-degrading bacteria, clostridia) for production of biofuels from biomass feedstocks. The ideal candidate will have expertise in modern molecular biology techniques, whole genome shuffling, DNA microarrays, proteomic and metabolic tools, transposon mutagenesis and high-throughput screening methods, to maximize the production of bioproducts and biofuels. (Hans Blaschek, Theme Leader)

BIOCOMPLEXITY

We seek a quantitative scientist with interests in evolution, systems biology, and ecosystem dynamics, and expertise in statistical physics as applied to biology. The successful candidate will join a multi-disciplinary group exploring collective effects in biology. Projects include the evolution of translation, the role of horizontal gene transfer in communities of microbes and phages, and the systems biology of cells and ecosystems. (Nigel Goldenfeld, Theme Leader)

REGENERATIVE BIOLOGY & TISSUE ENGINEERING

The Fellow will be involved in one or more of our multidisciplinary projects related to regenerative biology and harnessing the potential of adult/embryonic stem cells for tissue engineering applications. Of particular interest is leveraging theme expertise in biomaterials fabrication, drug delivery systems, microfluidics-based *in vitro* experimental platforms, and *in vivo* evolutionary biology and regeneration medicine studies. The ideal candidate will have experience in one or more areas of (stem) cell biology, induced pluripotent cell technology, biomaterials, microfluidics, and/or tissue engineering. (Paul Kenis, Theme Leader)

GENOMIC ECOLOGY OF GLOBAL CHANGE

The Fellow will be involved in a cross-disciplinary project investigating how changes in networks of genes affect ecosystem metabolism when challenged by elements of global change, including elevated atmospheric carbon dioxide and ozone, increased drought, and altered interactions with insect herbivores and plant pathogens. The ideal candidate will have a strong background in plant biology and a record of expertise in molecular biology, genomic ecology, physiology or modeling of gene networks or ecosystem function. The ability to work creatively and productively in a highly interdisciplinary and collaborative environment is essential. (Don Ort, Theme Leader)

ENERGY BIOSCIENCES INSTITUTE

The Energy Biosciences Institute (EBI) is an externally funded Theme within the IGB. It is the largest academia collaboration to date, currently receiving \$500 million over the next ten years and focusing on the development of second-generation biofuels intended to significantly slow the rate of global climate change. Its research ranges from systems biology of fermentative organisms to quantification of ecosystem services provided by new sustainable biofuel crops. The full range of research can be seen at www.energybiosciencesinstitute.org. We seek an outstanding candidate across these areas interested in applying genomic biology to understanding and developing opportunities for improving sustainable biofuel production. Research can be at any point in the supply chain from improving feedstocks and their environmental sustainability to producing fuel. The appointee will work in an inter-disciplinary laboratory of over 100 exceptional colleagues focused on this challenge. The appointment would also involve collaboration with our partners: UC Berkeley and BP. (Steve Long, Theme Leader)

MINING MICROBIAL GENOMES FOR NOVEL ANTIBIOTICS

The Fellow will be involved in a multi-disciplinary project aimed at the discovery, design, and development of phosphonic acid antibiotics. The ideal candidate will have a proven record of expertise in the general area of microbially produced natural products, with specific expertise in one of several disciplines. We are interested in candidates with previous experience in bacterial metabolism, bacterial genetics, molecular biology, biochemistry, enzyme evolution, metabolic engineering, organic synthesis, mass spectroscopy, bioinformatics and metagenomics. (Bill Metcalf, Theme Leader)

PRECISION PROTEOMICS

We seek an individual to develop next-generation technologies at the intersection of mass spectrometry and chemical biology. The successful Fellow will have advanced training in mass spectrometry of proteins (with emphasis on "Top Down" techniques and tandem mass spectrometry of high-mass ions), associated proteomics software, capillary separations, and cell culture. The Fellow will work in an interdisciplinary fashion with expert labs in single molecule fluorescence, natural products discovery, and high-throughput screening for anti-cancer compounds. (Neil Kelleher, Theme Leader)

GENOMICS OF NEURAL AND BEHAVIORAL PLASTICITY

We seek a biologist with strong bioinformatics skills and training in one or more of the following areas: evolutionary biology, neuroscience, animal behavior, molecular biology, or genomics. Applicants with expertise in both biology and bioinformatics will be strongly preferred. The successful candidate will join a multi-disciplinary team that is using genomics to identify both conserved and novel mechanisms of neural and behavioral plasticity in diverse animal systems. Fellows are expected to conduct research that contributes to the development of the theme's goals by integrating components from theme members' individual research programs. (Gene Robinson, Theme Leader)

HOST-MICROBE SYSTEMS

The Fellow will be responsible for developing DNA isolation, microbial isolation, 16S rRNA gene library construction and other metagenomic analysis techniques for surveying microbial content of the human and nonhuman primate vaginal and intestinal microbiomes. Additional responsibilities will include the development of microarray and other molecular biology techniques to examine microbial, metabolic, and immunologic contents, and performing analyses using bioinformatics and other computational and analytical methods. The ideal candidate will have a strong background in microbiology, biochemistry, or a related field with experience and expertise in molecular microbial ecology and bioinformatics and/or biostatistics. (Brenda Wilson, Theme Leader)

BUSINESS, ECONOMICS, AND LAW OF GENOMIC BIOLOGY

We seek an individual with training in economics, business, law, or strategy and with an interest in technology entrepreneurship, technology industries, and biotechnology. The Fellow will join a multi-disciplinary group that includes business, law, and technology experts; agricultural economics faculty; and personnel from the campus Office of Technology Management. Our theme is exploring issues in university-industry technology transfer, industry evolution, intellectual property protection, the competitive and cooperative dynamics for both entrepreneurial start-ups and existing corporations, the impact that globalization of biotechnology has on the evolution of industry, and the position of U.S. firms in the global marketplace. (Jay Kesan, Theme Leader)

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Through Inspiration, Discovery
King Abdullah University of Science and Technology

Faculty Positions in the Division of Chemical and Life Sciences and Engineering

King Abdullah University of Science and Technology (KAUST), located in Saudi Arabia, is an international graduate-level research university dedicated to advancing science and technology through bold and collaborative research and to addressing challenges of regional and global significance, thereby serving the Kingdom, the region and the world. KAUST faculty are engaged in such globally significant areas as Energy, Water, and Food. In addition, KAUST emphasizes research on the Environment and Red Sea and the discipline of Computational Science and Engineering serves as an enabling technology for all its research activities.

KAUST is located on the Red Sea in Thuwal (80km north of Jeddah). Newly opened in September 2009, KAUST is an independent and merit-based university and welcomes exceptional researchers, faculty and students from around the world. KAUST offers attractive base salaries and a wide range of benefits. Faculty will enjoy secure research funding from KAUST and have opportunities for additional funding through several KAUST provided sources and through industry collaborations. Further information about KAUST can be found at <http://www.kaust.edu.sa/>.

The Chemical Sciences program at KAUST invites applications for faculty positions at all ranks, which complements the KAUST ongoing research activities in Energy and Environmental sustainability and Catalysis thrusts. The successful applicant will be expected to develop a vigorous and world-class recognized research program, and enthusiastically join and/or embark in a collaborative and interdisciplinary research with one or more of KAUST's Research centers. The applicant must be eminently qualified to teach graduate courses in one of the chemistry core disciplines and may participate in Catalysis as well as materials-related Chemistry courses. The successful applicant is expected to supervise M.S. and Ph.D. students.

Potential applicants must possess a doctorate in Chemistry, Catalysis Science, Materials Science or closely related areas, and at the Assistant Professor rank preference will be given to applicants with postdoctoral experience in those areas.

Applications should include a curriculum vitae, statements on teaching philosophies and research plans with brief research proposals. Applicants must also arrange for letters of recommendation, at least 3 references for an Assistant Professor position and at least 6 references for an Associate or Full Professor position, to be sent by individuals who are knowledgeable of the applicants' professional work, directly to: ChemS@kaust.edu.sa

The review process of applications will begin immediately and applicants are strongly encouraged to submit applications as soon as possible.



Tenured and Tenure-Track Faculty Positions Available

FOX CHASE CANCER CENTER

CANCER BIOLOGY PROGRAM

We seek outstanding applicants with a Ph.D. and/or M.D. degree for appointment in our Cancer Biology Program. The successful applicant will receive a generous start-up package to develop a vigorous, extramurally-funded research program. We are particularly interested in collaborative researchers who will complement our current interests in genetics, epigenetics, cell signaling and stem cell biology. See: <http://www.fccc.edu/research/areas/CancerBiology/index.html>

Fox Chase, located in Philadelphia, offers a highly interactive, multidisciplinary environment, with over 50 active research laboratories on the 17-acre Fox Chase campus. Researchers are supported by state-of-the-art Core grant funded facilities, extramural and intramural postdoctoral training grants, a graduate training program, endowed fellowships, and an outstanding funding record for investigator-initiated grant applications. Applications will be reviewed starting April 15, 2010. To apply please send CV, description of research plans, and 3 letters of recommendation to: **Dr. Timothy Yen, Cancer Biology Program, c/o Anne Carson, Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111 (A_Carson@fccc.edu)**. Fox Chase Cancer Center is an Affirmative Action/Equal Opportunity Employer

Neutron Science Powder Diffraction Group Leader

Neutron Sciences Directorate at Oak Ridge National Laboratory invites applications for a Powder Diffraction Group Leader. ORNL is becoming the world's foremost center for neutron science. Research at ORNL encompasses the physical, chemical, materials, biological, and medical sciences and will provide opportunities for up to 2000 researchers each year from industry, research facilities, and universities all over the world.

The Powder Diffraction Group Leader will be responsible for ensuring efficient operation and availability of the neutron scattering instruments within the group and developing the instruments to meet the changing scientific needs of the neutron scattering community. The Group Leader encourages the development of the scientific programs of scientists in the group, seeking opportunities for establishing collaborations within and outside the group and participation in ORNL and external funding initiatives.

The successful candidate will have an international reputation and excellent track record, with at least ten years of specialized experience in the use and development of diffraction techniques for materials science research.

For more information about the position or to apply visit:
http://jobs.ornl.gov/neutron_science.shtml

neutrons.ornl.gov

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The University of Vienna, one of Europe's oldest universities, is Austria's leading research institution. Here more than 6,500 scientists and staff pursue cutting-edge research and teaching in 18 faculties offering a total of more than 200 different curricula.

One key strategic element toward the further strengthening of our position as a major European research-oriented university is a continuation of the recent series of high-caliber professorial appointments with international visibility.

In this context the University of Vienna intends to further strengthen its research activities and teaching profiles in Mathematics, Chemistry, and the Life Sciences by inviting applications for the following ten tenured full professorships*:



Faculty of Mathematics

Full Professor of Numerics of Partial Differential Equations
Full Professor of Stochastics
Full Professor of Dynamical Systems
Full Professor of Global Analysis/Differential Geometry
Full Professor of Mathematics and Biology



Faculty of Chemistry

Full Professor of Materials Chemistry
Full Professor of Separation Technologies and Bioanalytics
Full Professor of Organic Synthesis: Natural Products, Methods
Full Professor of Computational Chemistry – Theoretical Chemistry/Scientific Computing



Faculty of Life Sciences

Full Professor of Public Health Nutrition

Closing date for applications is May 31, 2010.

**To increase the fraction of women among its professoriate the University of Vienna particularly invites applications from women. When two applicants of opposite sex are found equally qualified, the woman will be preferred.*



For the full application form, including employment conditions, please see:

<http://jobcenter.univie.ac.at>



Center for Immunology and Microbial Disease Albany Medical College

Faculty Position

The Center for Immunology and Microbial Disease at Albany Medical College invites applications for a tenure-track faculty position from individuals who have a doctoral degree, postdoctoral experience, and demonstrated research productivity. Those with an interest in microbiology and/or host-pathogen interactions are particularly encouraged to apply. The successful candidate will be expected to establish an independent, extramurally funded research program and participate in the teaching of medical and graduate students. The basic science departments at Albany Medical College are organized as interdisciplinary research centers and the Center for Immunology and Microbial Disease has a focus on microbial pathogenesis and immune defense, particularly as related to biothreat agents and emerging infections. Faculty at the Albany Medical College receive competitive salaries, attractive start-up packages, and access to the Center's ABSL-3/BSL-3, Microbiology and Immunology Core Labs. In addition, we have established a close relationship with the New York State Department of Health Wadsworth Laboratories, providing a diverse environment that is rich in infectious disease expertise. Albany Medical College is located in a mid-sized city within the upstate New York Capital Region, and has easy access to Boston, New York City, and the Adirondack Mountains.

Applicants should send their curriculum vitae, a statement of research plans, and three letters of reference to:

Faculty Search Committee
Center for Immunology & Microbial Disease
Albany Medical College
47 New Scotland Avenue, MC-151
Albany, NY 12208

For further information about the Center, visit <http://www.amc.edu/Research/IMD/index.html>.

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Women and minorities are encouraged to apply.*



TEXAS TECH UNIVERSITY
Edward E. Whitacre Jr.
College of Engineering



The Maddox Chairs in Energy at Texas Tech University

The Edward E. Whitacre Jr. College of Engineering at Texas Tech University is committed to leveraging these **two exceptionally large endowed chairs at over \$7 million each**, to become one of the nation's leaders in finding solutions to the world's energy challenges. The college is seeking world-class researchers in solar and sustainable energy as candidates for the Maddox Chairs.

Donovan Maddox Distinguished Engineering Chair in Solar Energy

Candidates are expected to have national and international reputation in solar energy based on research publications. In addition, a record of acquiring external resources to support research, team building, and mentoring of associates and graduate and undergraduate students is necessary. The holder of the Donovan Maddox Chair will be expected to not only bring his or her own research activities to the Whitacre College of Engineering, but also to build a collaborative community of scholars at Texas Tech dedicated to solar energy research, thereby building a world-class research program. The appointment will be as a full professor in the Whitacre College of Engineering.

Jack Maddox Distinguished Engineering Chair in Sustainable Energy

Candidates with exceptional and diverse backgrounds in energy sciences and engineering are sought for this endowed position. The successful candidate will demonstrate a national and international reputation for contributions to the solution or advancement of the state of the art on a variety of research issues in the sustainable energy fields including energy efficiency, biofuels, wind power, tidal power, geothermal, and energy storage. The successful candidate, along with the Donovan Maddox Chair in Solar Energy, will set the tone, vision, and the path in order to build a nationally and internationally recognized program at Texas Tech University in sustainable energy research. The appointment will be as a full professor in the Whitacre College of Engineering.

Screening will begin upon the receipt of applications and will continue until the position is filled. Candidates names will not be made public until the final stages of the search. Curriculum vitae and the names and contact information of at least four references should be submitted at www.coe.ttu.edu/maddox. To nominate a colleague for these chairs, visit www.coe.ttu.edu/maddox. Nominations can be made anonymously.

Questions about the Jack Maddox or Donovan Maddox Chairs should be directed to:

Jack Maddox and Donovan Maddox Search Committees
Texas Tech University | Whitacre College of Engineering
Box 43103 | Lubbock, Texas 79409-3013 | engineering@coe.ttu.edu | 1.800.528.5583

Texas Tech University | Whitacre College of Engineering
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The Professorship of Experimental Astrophysics

The Board of Electors to the Professorship of Experimental Astrophysics invite applications for this Professorship from persons whose work falls within the general field of the Professorship to take up appointment on 1 October 2010 or as soon as possible thereafter. We interpret experimental astrophysics in a broad sense encompassing the field from astronomical instrumentation through the design and specification of new telescopes and techniques to scientific leadership associated with new telescopes and facilities.

Further information is available at:

www.admin.cam.ac.uk/offices/academic/secretary/professorships/ or contact the Academic Secretary, University Offices, The Old Schools, Cambridge, CB2 1TT (email: ibise@admin.cam.ac.uk) to whom a letter of application should be sent, together with details of current and future research plans, a curriculum vitae, publications list and form PD18 (parts I and III only) with details of two referees, so as to reach him no later than 21 May 2010.

Informal enquiries may be made to
Professor Peter Littlewood, Head of the
Department of Physics, telephone: +44(0)1223 337429
(e-mail: hod@phy.cam.ac.uk)

The University is committed to Equality of Opportunity.



Faculty Positions available at the
State Key Laboratory of Experimental Hematology,
Institute of Hematology, Tianjin, P. R. China

Multiple faculty positions at associated investigator or investigator level are available at the State Key Laboratory of Experimental Hematology (SKLEH), China. SKLEH is housed at the Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China. SKLEH is the only national laboratory designated by the Chinese government focusing on experimental hematology and pathogenesis of blood diseases with ample financial support. SKLEH is a leader in basic and applied hematology with a broad research scope focusing on stem cell biology and its applications; molecular basis of diseased hematopoietic microenvironment; functional genomics and molecular targets for hematopoietic malignancies, etc. The laboratory maintains state-of-the-art core technology platforms including human hematopoietic cell and tissue bank, stem cell isolation, cell imaging, animal facility, functional genomics core and antibody production.

A Ph.D with relevant postdoctoral training, a strong publication record and research interests that fit or complement the current research scope of SKLEH are required. An attractive package including strong startup research funding, technical supporting staffs, graduate student recruiting, individualized compensation plan and housing assistance will be provided. For a full position description and application procedures, visit <http://www.chinablood.com.cn/sygf/>.

Interested candidates should send a letter containing the position they are interested in, a description of their career goals and research interests, their curriculum vitae, and names and addresses of three references to Dr. Weiping Yuan, Assistant Director of the SKLEH at wpyuan@ihcams.ac.cn or Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, 288 Nanjin Road, Tianjin 300020, China. Applications will be reviewed upon receipt and searches will be continued until all positions are filled.



Faculty and Research Positions in Ocean Sciences and Engineering

The Red Sea Research Center at the King Abdullah University of Science and Technology (KAUST) invites applications from scientists and engineers interested in joining in developing an integrated understanding of the Red Sea marine ecosystem, from the molecular/-omic level to the physical and chemical environment in which the whole ecosystem exists. We are seeking creative and independent individuals with specific interests in Physical, Chemical and Biological Oceanography, Marine Biology/Marine Ecology, Genomics, and Ecosystem Modeling. We are particularly interested in those who will make effective use of the unique access to the Red Sea region that KAUST can provide.

We seek colleagues to join our Marine Science & Engineering faculty at the Assistant, Associate, or full Professor level with expertise in areas of Ocean Physics and Modeling (FA1), Coupled Bio-physical Oceanography (FA2) (two additional faculty positions may be available). All expressions of interest should indicate the potential to complement and strengthen our existing expertise and utilize Red Sea Research Center and other KAUST facilities. Several research staff positions are also available. Potential areas of expertise we seek to fill include, but are not limited to, Research Scientist in the area of Bioinformatics (RS1), Research Technician in the area of PCR/genetics facilities management (RS2), Postdoctoral Researcher in the area of fisheries research (RS3), Postdoctoral Researcher in the area of acoustic and/or archival tagging of fishes (RS4).

Our graduate program in Marine Science encompasses two tracks – Marine Biology & Conservation and Ocean Physics & Modeling. Appointments to the Marine Science faculty will require involvement in the Marine Science academic program, while those on the Research Staff do not.

KAUST has been established as a world-class international graduate-level science and technology research university dedicated to inspiring a new age of scientific achievement in the Kingdom that will also benefit the region and the world. The KAUST mission emphasizes research on applications of science and technology to problems of human need, social advancement, and economic development, in collaboration with leading universities around the world. The overarching themes for KAUST in the coming decade are Energy, Water, Food, Red Sea and Environment, including high performance computing as a cross-disciplinary enabling technology. KAUST is dedicated to a respect for diversity and the highest standards of merit-based opportunity, and seeks the finest students and faculty without regard to race, gender or religious belief. The campus is located in Thuwal, 80 km north of Jeddah, Saudi Arabia. KAUST has established advanced research centers that focus on interdisciplinary problems as the central organizing unit, and offering only graduate (M.S. and Ph.D.) degrees. KAUST has three academic divisions: Chemical and Life Sciences, Physical and Environmental Sciences and Engineering, and Mathematical and Computer Sciences and Engineering. KAUST offers unparalleled analytical, research, and computing facilities, with ready access to the Red Sea environment.

Those interested in pursuing this unique opportunity should send a CV (including publication lists and contact information for at least three potential references) and a statement of research interests. Potential faculty members should include a statement of teaching interests. Send these materials and any additional enquiries to MarSE@kaust.edu.sa. Further details can be found on the University's website: <http://www.kaust.edu.sa>



THE UNIVERSITY OF
WARWICK

Neuroscience Research Opportunities in Singapore

The Nanyang Technological University (NTU), Singapore, and the University of Warwick, UK, are seeking two outstanding post-doctoral researchers for tenure-track positions to lead a new international research programme in advanced neuroscience research to analyse neuron-glia interactions via optogenetic methods.

NTU is an internationally reputed research-intensive university with globally acknowledged strengths in science, engineering and technology. The University of Warwick is one of the UK's leading universities with an acknowledged reputation for excellence in research and teaching, innovation, and links with business and industry.

These tenure-track appointments will come with an initial contract of 5 years and will be joint faculty positions between NTU and the University of Warwick. The appointees will be based in Singapore at the internationally-renowned Biopolis research complex, and will be part of the contingent of neuroscientists working in the Neuroscience Research Partnership who have expertise in the use of optogenetic methodology and cellular imaging to study neuronal function and circuitry.

The two new faculty members will be expected to spend periods in the UK working with colleagues in the University of Warwick. The research programme will have two coordinated thrusts, one in molecular biology and one in glial cell biology, aimed at developing and exploiting optogenetic tools for the manipulation of glial cell function in the brain.

Successful candidates will have a record of research excellence in a relevant field and should have completed at least one post-doctoral research period.

An attractive laboratory start-up and remuneration package is available.

For enquiries, contact Mark Featherstone at NTU (msfeatherstone@ntu.edu.sg) or Nick Dale at the University of Warwick (n.e.dale@warwick.ac.uk). Applicants should email a cover letter, detailed curriculum vitae and brief description of their research plans to msfeatherstone@ntu.edu.sg. For more information about the two universities, visit www.ntu.edu.sg and www.warwick.ac.uk. For more information on the positions, visit <http://www.ntu.edu.sg/ohr/Career/SubmitApplications/Pages/Faculty.aspx>.

Application Deadline: **31 May 2010**

www.ntu.edu.sg

FACULTY POSITIONS IN MICROBIAL PATHOGENESIS

The Department of Microbiology & Immunology at New York Medical College invites applications for tenure track faculty positions in the broad area of Microbial Pathogenesis. It is expected that appointments will be made at the Assistant or Associate Professor levels. Our focus will be on candidates who will employ molecular approaches to pathogenesis/immunopathogenesis of microbial infections. Areas of particular interest include, but are not limited to, newly emerging pathogens and cellular microbiology. We are seeking individuals with a commitment to establish a vigorous, independent research program, to participate in teaching of medical and graduate students and to interact with other members of the department and medical school faculty. The search process will begin immediately and will continue until the available positions are filled.

Interested individuals should submit curriculum vitae, selected reprints and a description of current and future research plans, and arrange to have three letters of recommendation sent to:

Dr. Ira Schwartz, Chairman
Department of Microbiology & Immunology
New York Medical College
Valhalla, NY 10595
E-mail: schwartz@nymc.edu

New York Medical College is an equal opportunity employer that seeks a faculty as diverse as its student body and the communities it serves.



Accredited by the Council on Education or Public Health (CEPH).

EOE



Iowa Center for Muscular Dystrophy Research and Department
of Molecular Physiology and Biophysics
University of Iowa Carver College of Medicine

Faculty Positions Skeletal Muscle Biology and Disease

The Iowa Center for Muscular Dystrophy Research seeks outstanding candidates for tenure-track positions at any rank. Successful candidates are expected to establish independent laboratories focusing on skeletal muscle biology and disease. We are particularly interested in individuals who would complement existing strengths in the Center.

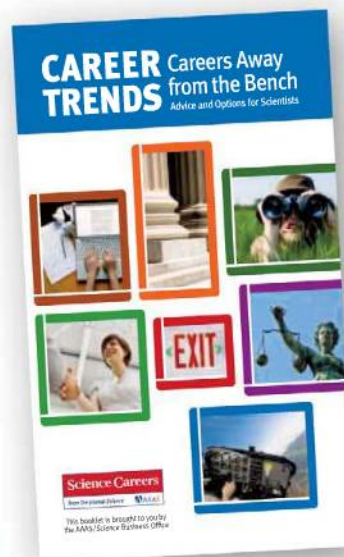
These positions feature outstanding research space with state-of-the-art shared instrumentation, as well as substantial startup funds for equipment, personnel support and supplies. The University of Iowa is located in Iowa City, an affordable college community with many cultural amenities.

All applicants must have a relevant doctoral degree, productive research experience, and a strong record of research accomplishment. Candidates are judged on their potential to initiate and maintain a creative, independent research program, and their desire to train students and postdoctoral fellows.

To apply for this position, please visit the University of Iowa website at <http://jobs.uiowa.edu/faculty/view/57388>. Applicants should include a CV, letter of interest, and the names of three references. Consideration of completed applications will begin on **February 15, 2010**. Questions may be directed to **Dr. Kevin P. Campbell, Director of the ICMDR** at kevin-campbell@uiowa.edu.

The University of Iowa is an Equal Opportunity/Affirmative Action Employer. Women and minorities are strongly encouraged to apply.

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From the Journal Science AAAS

The DZNE advertises a position as

Head of Strategy (m/f)

– Code 1005/2010/1 –

The DZNE is a newly created center of excellence within the Helmholtz Association that will perform translational research on Neurodegenerative Diseases. The center includes a major site in Bonn and additional sites in Goettingen, Magdeburg, Muenchen, Rostock/Greifswald, Tuebingen and Witten/Herdecke.

The Head of Strategy will help developing and implementing short- and long-term strategic scientific plans in support of the scientific director. He or she will coordinate with the Executive Board (scientific and administrative director) the evaluation of the science progress and the new developments of the whole DZNE. The candidate will provide strategic scientific support to all DZNE partner institutes in order to realise and combine translational research throughout the DZNE. The candidate will provide input on new scientific developments worldwide in the field of neurodegenerative diseases.

Qualifications

- The candidate should have at least ten years experience within biomedical research. Candidates with a strong neuroscience background and a strong background/experience in research management and/or funding will be prioritised.
- The right candidate should demonstrate ability to work effectively in a team-based environment.
- Strong verbal and written communication skills in English are required. Knowledge of German would be preferable.

Employment, payment and social benefits are determined by the Public Sector Collective Agreement (Tarifvertrag für den öffentlichen Dienst – TVöD). The job location is Bonn, Germany. The DZNE seeks to increase the number of female personnel in those areas where they are underrepresented and therefore explicitly encourages women to apply. The DZNE is committed to employing more disabled individuals and especially encourages them to apply.

Applications should be sent to Frau Nicole Friese, Deutsches Zentrum für Neurodegenerative Erkrankungen e. V., Ludwig-Erhard-Allee 2, D - 53175 Bonn or via e-mail to application@dzne.de. Deadline for the application is **16.04.2010**.

The Scottish Universities Physics Alliance

The Scottish Universities Physics Alliance is an exciting, vibrant, research led academic community offering opportunities to work with leading international academics whose visions are shaping tomorrow's world.



Chief Executive Officer

Salary in the professorial range

SUPA is an alliance of eight universities aimed at developing physics in Scotland into a world leading force through pooling resources and planning research strategy nationally. The formation of SUPA has been boosted by a £48M research investment programme over the next seven years funded by the Scottish Funding Council and the constituent universities.

SUPA seeks a scientist of international standing to lead the Alliance, following the retirement of Prof Ian Halliday CBE FRSE, who has been its Chief Executive Officer (CEO) since 2005. The CEO will promote the SUPA branding of Scottish physics, develop its Scotland-wide Graduate School and establish a Scotland-wide Knowledge Transfer programme that maximises the impact of SUPA's research.

You should have an international track record in any area of Physics or Astronomy research relevant to the SUPA universities, as well as experience and proven success in science management and policy at a senior level. Salary will be at a competitive level for such a senior position, and the post could be held at any one of the eight SUPA Universities. The appointment, which may be full-time or part-time, will be for four years in the first instance, with the possibility of extension for a further four years.

Applications are being handled by the University of Edinburgh on behalf of the consortium. More information about SUPA can be found at <http://www.supa.ac.uk>. Members of SUPA are The Universities of Aberdeen, Dundee, Edinburgh, Glasgow, St Andrews, Strathclyde, the West of Scotland, and Heriot-Watt University.

To apply online or view more job opportunities, visit our website. Alternatively, telephone the recruitment line on 0131 650 2511. Ref: 3012560S. Closing date: 30 April 2010.

Committed to Equality and Diversity
The University of Edinburgh is a charitable body, registered in Scotland, with registration number SC005336.

www.jobs.ed.ac.uk

Nanotechnologies for Biomolecular Physics



Eindhoven University of Technology (The Netherlands) invites candidates to apply for the position of:

Assistant/Associate Professor (tenure track)

in the focus area Nanotechnologies for Biomolecular Physics.
For more information:
www.phys.tue.nl/hrm

www.tue.nl/jobs

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*Your Future
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Science Careers

From the journal *Science* AAAS

ScienceCareers.org



ASSISTANT DIRECTOR OF RESEARCH RESOURCES

The Southwest National Primate Research Center (SNPRC), www.snprc.org, located at the Southwest Foundation for Biomedical Research (SFBR), in San Antonio, TX., invites applications and nominations for the position of Assistant Director of Research Resources. The Assistant Director of Research Resources is a key member of the SNPRC's scientific administrative staff with primary responsibility for supporting the research activities of the SNPRC.

Qualified applicants must have a doctoral degree (e.g., Ph.D., D.V.M., M.D., etc.) and at least 10 years of relevant professional experience in research or administration at a major university or nonprofit research institution. Have demonstrated ability to function independently, excellent judgment, outstanding managerial and organizational capabilities, strong interpersonal skills, and the ability to work well with scientists in developing research programs and structuring research support.

Interested individuals should send a letter of interest, resume, and names and contact information for three references to: **Dr. Thomas Folks (210-258-9889)**, Associate Director of Research Resources, SNPRC, c/o HR Director, SFBR, P.O. Box 760549, San Antonio, Texas 78245; www.sfbr.org.

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From the journal *Science* AAAS



Associate Editor

Science and AAAS seek a talented scientist to serve as an Associate Editor for our new interdisciplinary journal, *Science Translational Medicine*.

This position is designed for an individual with broad interests, a lively curiosity, and experience with cutting-edge research in at least one, but preferably more than one, biomedical or clinical research field. To round out our editorial team, we would like our new Associate Editor to have expertise in immunology (vaccines and autoimmune disease especially welcome) or bioengineering (devices, tissue engineering and stem cells are areas of preference).

Responsibilities include, but are not limited to:

- Judge the scientific value of research;
- Foster relationships and communication with the scientific community through literature reviews, meetings and professional contacts;
- Manage the review, selection, and editing of submitted manuscripts;
- Select reviewers for submitted manuscripts;
- Discuss and make recommendations regarding manuscripts and reviews with other staff, advisers, authors;
- Write summaries of research results for publication;
- Guide authors on manuscript revisions;
- Edit the manuscripts for scientific content and style before and after revisions;
- Follow the manuscript through production process to ensure material is published in a timely manner; and
- Travel to scientific meetings.

The minimum qualifications to be competitive and considered for the position are:

- Mastery of a professional field typically acquired through completion of a doctoral degree in at least one biomedical or clinical research field;
- 3-5 years experience, including post-doctoral research experience and multiple publications;
- Ability to work constructively as a member of a team;
- Experience with cutting-edge research in one of the fields mentioned above;
- Comprehensive knowledge of scientific research methods in order to discuss technical issues with authors; and
- Exceptional written, communication, and listening skills in order to communicate with authors and reviewers in evaluating, editing and modifying manuscripts.

Previous editorial experience is not required.

If you would like to be a member of the AAAS team, please visit our Job Information web-site at <http://www.aaas.org/careercenter/employmentataaas/> to get more information and to apply online today.

AAAS is an Equal Opportunity Employer.



Faculty Position in Civil and Environmental Engineering at the Ecole polytechnique fédérale de Lausanne (EPFL)

EPFL's School of Architecture, Civil and Environmental Engineering seeks a **Tenure-Track Assistant Professor of Geo-Engineering focusing on CO₂ and Sequestration**.

The future success of CO₂ sequestration in deep, geologically secure formations, either on-shore or off-shore, remains uncertain due to numerous technical, engineering and scientific challenges. The challenges include, basic understanding of the physico-mechanical and biogeochemical processes involved, the role of geological controls on long term storage, methods of locating sites and monitoring their performance, technology development, and combined storage and hydrocarbon recovery.

We seek applications from highly qualified engineers and engineering scientists committed to a career in research and teaching. Applications are welcomed from all the disciplines pertinent to CO₂ sequestration, including, but not limited to, geological, geochemical, environmental and civil engineering. The successful applicant will have potential for developing a research profile characterized by novel accomplishments, competitive grant funding and an interdisciplinary, collaborative vision. A broad vision of geo-engineering is sought, as is the ability to collaborate across disciplines.

Successful candidates are expected to initiate independent research programs and participate in undergraduate and graduate teaching. Substantial start-up resources will be available. We offer internationally competitive salaries and benefits

Applications should include a résumé with a list of publications, a concise statement of research and teaching interests, and the names and addresses (including e-mail) of at least four referees. Applications should be submitted electronically to <http://enac.epfl.ch/page24888.html> by **15 May 2010**, when formal screening of applications will begin.

Informal enquiries may be made to:
Professor D. Andrew Barry
andrew.barry@epfl.ch

Additional information about EPFL is available at <http://www.epfl.ch>, <http://enac.epfl.ch>.

Ecole polytechnique fédérale de Lausanne is an equal opportunity employer.

Woman candidates are particularly encouraged to apply.

Career opportunities in the tropics

Lecturer/Senior Lecturer – Physiology

Ref. No. 1050 - Townsville, Queensland, Australia

The School of Veterinary and Biomedical Sciences is seeking a Physiologist to join the Discipline of Physiology and Pharmacology within the Faculty of Health and Molecular Sciences. Physiology is taught in a broad range of courses including Integrated Medical and Veterinary Curricula, Rehabilitation Sciences, Biomedical Sciences, Sport and Exercise Science, Medical Laboratory Science, Pharmacy, Nursing, and Dentistry. The appointee will have the opportunity to work with existing academic strengths in the development and delivery of a range of degree programs and to pursue research opportunities relevant to the region. The co-location and collaboration between the Schools of Medicine and Dentistry; Public Health, Tropical Medicine and Rehabilitation Sciences; Pharmacy and Molecular Sciences; and the School of Veterinary and Biomedical Sciences at JCU presents unprecedented research opportunities.

Employment Type: Appointment will be full-time on a continuing basis subject to a probationary period.

Salary: Lecturer - Academic Level B - A\$69,689 - A\$82,461 per annum or Senior Lecturer - Academic Level C - A\$85,013 - A\$97,784 per annum. Level of appointment and commencing salary will be in accordance with qualifications and experience. Benefits include generous employer superannuation contribution and attractive options for salary packaging.

Applications close on 30 April 2010.

For more information go to: www.jcu.edu.au/jobs
enter the Reference Number in the search field and follow the links.

www.jcu.edu.au/jobs



10th Annual Conference of Science & Technology in Society

A venue for all graduate students from Science & Technology Policy (STP), Science & Technology Studies (STS), and related fields

**April 9-11, 2010
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Google Vice President & Chief Internet Evangelist

Dr. John P. Holdren:

Director OSTP

Dr. Margaret Hamburg:

Commissioner FDA

4th speaker TBA

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to register go to
<http://www.stglobal.org>**



Call for Theme Proposals for EUROCORES research programmes

The European Science Foundation is looking for new ideas for collaborative research at the European level. We invite well developed theme proposals for new EUROCORES Programmes.

→ Deadline **Friday 21 May 2010** (noon CET)

Please see www.esf.org/EUROCORES for application and eligibility criteria, and other information on the call.

The EUROCORES Scheme

The European Collaborative Research (EUROCORES) Scheme enables researchers in different countries to collaborate in tackling the questions that need international scale and scope for top class science in a global context. The scheme is scientist-led, providing an exceptional opportunity for scientific communities from Europe and beyond to submit their own ideas for medium- to large-scale multinational collaborative research programme themes across all disciplines. Successful theme proposals selected through this call will request specific project proposals in December this year.

The European Science Foundation

The ESF provides a platform for 79 Member Organisations from 30 countries to advance science and explore new directions for research at the European level. ESF is an independent non-governmental organisation, devoted to the coordination, implementation, networking and science policy development in the basic sciences. The EUROCORES Scheme is part of the ESF contribution to the European Research Area. The research, networking and coordination costs of EUROCORES programmes are covered by participating national funding organisations.

For further inquiries contact the EUROCORES Scheme at eurocores@esf.org

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Science/UCSF Biotech Industry Career Day

1 April 2010
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Genentech Hall
10:00 am–4:30 pm

Science and UCSF are teaming up to bring you a unique biotech day that includes five career development workshops. Visit Genentech Hall on the the Mission Bay campus for a chance to get valuable advice from career experts, and to meet face to face with recruiters from some of the world's top scientific organizations. For details, visit sciencecareers.org/ucsf

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AAAS

POSITIONS OPEN



FACULTY POSITION IN MICROBIOLOGY

The department of Health Sciences in the College of Public Health at East Tennessee State University invites qualified applicants for a 12-month, tenure-track faculty position at the level of **ASSISTANT/ASSOCIATE PROFESSOR** beginning fall 2010 or spring 2011. We seek a broadly trained **MICROBIOLOGIST** with demonstrated ability to teach both an upper-level course in area of expertise, as well as mid and introductory level classes in microbiology. Development of a strong, extramurally fundable research program with undergraduate and graduate (M.S.) student participation is expected. Startup funds and laboratory space are available. A Ph.D. in microbiology, strong publication record, and postdoctoral experience are preferred. Applicants should electronically submit curriculum vitae, description of research interests and goals, statement of teaching interests and philosophy and contact information for three references to Chair of Health Sciences Faculty Search at e-jobs (as per instructions) at [website: https://jobs.etsu.edu/applicants/jsp/shared/application/ChooseApplicationType_css.jsp](https://jobs.etsu.edu/applicants/jsp/shared/application/ChooseApplicationType_css.jsp).

FACULTY POSITION IN MICROBIOLOGY Center for Immunology and the Infectious Diseases Corridor University of Minnesota

The Department of Microbiology and the Center for Immunology announce a partnership-recruitment under the Corridors of Discovery initiative at the University of Minnesota Medical School and Academic Health Center. We invite applications for a faculty position to be filled at the tenure-track **ASSISTANT PROFESSOR** level in the area of the immune response to chronic viral infections. The successful applicant will be expected to establish a productive, federally funded, independent research program in the Center for Immunology, and to contribute to teaching in the Department of Microbiology. Information about current research activities can be found at [websites: http://www.microbiology.med.umn.edu](http://www.microbiology.med.umn.edu), <http://www.immunology.umn.edu/>, and <http://www.ahc.umn.edu/research/corridors/infectiousdisease/>.

Minimum qualifications: Ph.D. in microbiology or immunology, M.D. or D.V.M., and applicable postdoctoral or faculty experience. To apply, please submit curriculum vitae and concise (no more than two pages) summary of research interests to **requisition number 165428** at [website: http://employment.umn.edu](http://employment.umn.edu) no later than May 1, 2010. Please also arrange to have three letters of recommendation electronically sent to e-mail: microbiology@umn.edu or sent by surface mail to: **Chronic Infections Search Committee, Department of Microbiology, University of Minnesota, MMC 196, 420 Delaware Street S.E., Minneapolis, MN 55455**. Top candidates will be invited for a seminar/interview as a component of the selection process.

The University of Minnesota shall provide Equal Access to and Opportunity in its Programs, Facilities, and Employment without regard to race, color, creed, religion, national origin, gender, age, marital status, disability, public assistance status, veteran status, sexual orientation, gender identity, or gender expression.



THE UNIVERSITY of TEXAS
MEDICAL SCHOOL at HOUSTON

A part of The University of Texas Health Science Center at Houston

A **POSTDOCTORAL POSITION** is immediately available to explore the mechanisms behind how deep brain stimulation (DBS) works as a therapy for various neurologic disorders. Work will be performed on rodent models with electrophysiological approaches. We want an extremely self-motivated scientist with experience in intra- and extracellular recordings and patch clamp techniques to explore this hot topic area.

Interested parties should contact e-mail: albert.j.fenoy@uth.tmc.edu with references.

POSITIONS OPEN



RESEARCH INSTRUCTOR IN BIOORGANIC CHEMISTRY AND MOLECULAR PHARMACOLOGY Washington University School of Medicine

The Division of Bioorganic Chemistry and Molecular Pharmacology in the Department of Medicine at the Washington University School of Medicine seeks candidates for a Research Instructor position. The successful applicant will be expected to develop and perform detailed proteomic and metabolomic analyses using liquid chromatography/mass spectrometry-based methods to determine alterations in protein composition and posttranslational modifications that mediate the sequelae of diabetic heart disease. Applicants should have a Ph.D. in chemistry with a focus on mass spectrometry as well as at least two years of experience in proteomics and/or metabolomics at the postgraduate level. Experience with electrospray ionization and high performance liquid chromatography are essential and additional experience with matrix-assisted laser desorption/ionization is preferred. Experience in other aspects of chemistry including the enrichment sciences, organic chemistry, or biochemistry will be viewed favorably. We are especially interested in individuals who are committed to research in a fast moving research environment where multiple new methods are developed and directly applied to human disease.

Applicants should send a letter of interest, curriculum vitae including complete publication list, and contact information (address, e-mail, and telephone) to: **Dr. Richard W. Gross, Professor and Chief, Division of Bioorganic Chemistry and Molecular Pharmacology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8020, St. Louis, MO 63110**. We would prefer electronic submission to e-mail: rgross@wuchem.wustl.edu. Applications will be accepted until the position is filled. *Equal Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.*

RESEARCH FACULTY POSITION

The University of Rochester
School of Medicine and Dentistry
Division of Endocrinology and Metabolism

The Division of Endocrinology and Metabolism at the University of Rochester School of Medicine is seeking new Research Faculty members of all ranks. Candidates must have an M.D. or Ph.D. and should be performing endocrine-related basic or translational research. The Division is under new leadership, is moving into newly renovated space, and is seeking to rapidly expand its academic programs. Thus, the University of Rochester offers an exciting opportunity for research scientists interested in endocrinology. Please electronically send curriculum vitae and cover letter to **Stephen Hammes, Chief, Division of Endocrinology and Metabolism, University of Rochester School of Medicine, e-mail: stephen_hammes@urmc.rochester.edu**.

The University of Rochester has a strong commitment to principles of diversity and, in that spirit, actively encourages applications from groups underrepresented in higher education.

TENURE-TRACK NEURODEGENERATION RESEARCH POSITION

Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh, seeks an established, full-time faculty member to conduct laboratory research on neurodegenerative diseases. Applicants should have an M.D. or Ph.D. degree, a track record of extramural funding, and must be eligible to work in the U.S.A. Generous startup package is available, and there is a possibility of an endowed chair. Applicants should electronically submit curriculum vitae and a brief statement of research interest to **J. Timothy Greenamyre, Chair, Search Committee, Department of Neurology, e-mail: neurologyinfo@upmc.edu**.

The University of Pittsburgh is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN



UNIVERSITY OF MARYLAND
SCHOOL OF MEDICINE

The University of Maryland School of Medicine has **POSTDOCTORAL FELLOWSHIP POSITIONS** starting July 1, 2010, in our NIDDK-sponsored T32 training program, centered in the Division of Gastroenterology and Hepatology. Applicants (M.D., M.D.-Ph.D., Ph.D.) must be U.S. citizens or permanent residents committed to an academic career in gastrointestinal or liver research. Information regarding research tracks/mentors available at [website: http://www.umm.edu/gi/t32_training_grant.htm](http://www.umm.edu/gi/t32_training_grant.htm). Interested applicants electronically submit cover letter indicating career goals, names and e-mail addresses for three references, and curriculum vitae to **Jean-Pierre Raufman, M.D., e-mail: jraufman@medicine.umaryland.edu**.

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Promoting your ambition is what we do. We're your catalyst for connecting with the industry's top employers. We're the experts and source for accessing the latest and most relevant career information across the globe.

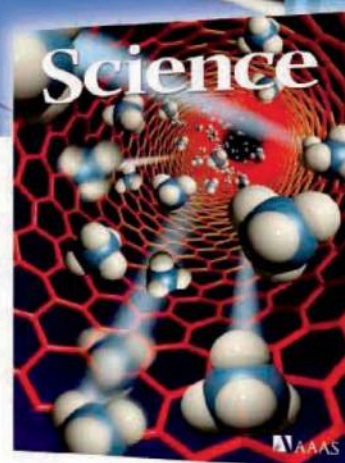
Our newly designed website offers a set of tools that help you discover career opportunities and your personal potential. Whether you're seeking a new job, career advancement in your chosen field, or ways to stay current on industry trends, *Science Careers* is your catalyst for an accelerated future.

Improved Website Features:

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- » Improved Resume Uploading
- » Content Specific Multimedia Section
- » Facebook Profile

Job Search Functionality:

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- » Track Your Activity
- » Search by Geography
- » Enhanced Job Sorting



Your Future Awaits.

Science Careers

From the journal *Science*



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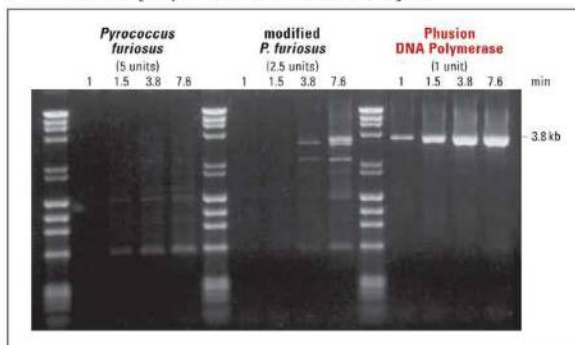


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